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Ion-pair reversed-phase liquid chromatography–quadrupole-time-of-flight and triple-quadrupole–mass spectrometry determination of ethyl sulfate in wastewater for alcohol consumption tracing

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ABSTRACT

Ethyl sulfate (EtS) is excreted in urine as a minor metabolite (0.010–0.016% on molar basis) after intake of alcoholic beverages, being a convenient biomarker for ethanol tracing after its determination in sewage. In this work, a new method for the direct determination of EtS in wastewater by liquid chromatography–(tandem) mass spectrometry (LC–MS(/MS)) has been developed. Different LC columns, mobile phases, and detection systems have been tested. Convenient retention by ion-pair reversed-phase LC was achieved by addition of 50 mM tetrabutylamonium bromide to the sample. Also, a triple-quadrupole (QqQ) instrument and a quadrupole time-of-flight (QTOF) system were compared. The repeatability of both systems and linearity was comparable, with RSD $\leq$ 10% in sewage samples. The QqQ instrument provided a better limit of detection (LOD = 0.1 $\mu g\,L^{-1}$) than the QTOF system LOD (0.2 $\mu g\,L^{-1}$). However, the LOD of this last instrument was still good enough for wastewater concentrations, while avoiding problems with interferences on the QqQ not permitting positive identification with this last system. The stability of EtS was tested and it has proven to be stable in wastewater for at least one week at room temperature and more than one month at −20 °C. The application of the method to samples collected during a week in a Galician (NW Spain) city showed EtS concentrations between 4 and 12 $\mu g\,L^{-1}$. This translated into a per capita consumption of pure ethanol in the range from 9 to 24 mL day^{-1} inh^{-1}, observing an increase during the weekend compared to weekdays.

1. Introduction

Abuse of alcoholic beverages is a sociological phenomenon all over the world. Actually, ethanol is the most commonly used substance of abuse [1,2]. Health problems created by excessive ethanol consumption have been rapidly growing in many countries [1,3]. Moreover, in the last years, the binge drinking phenomenon has increased over the weekend periods [1]. Besides, the abuse of alcohol, and its combination with other drugs (cocaine, cannabis, amphetamines, heroin, etc.) has incalculable societal consequences, such as health treatment costs, higher incidence of criminality, economic damage and traffic accidents [1,4].

The average alcohol consumption in a population can be deduced from alcohol sales statistics, but such data are not always available or reliable [5]. Similar uncertainty also applies to consumption data obtained from interview and questionnaire-based information from population [6]. Therefore, additional methods are required in order to determine the level of consumption in a certain population. A possible procedure consist in the so-called *sewage epidemiology* approach, consisting in tracing a marker (normally a metabolite) in wastewater, and then use pharmacokinetic, wastewater flows and population data to derive the consumption of a determinate substance. Such approach is now well established in the determination of illicit drugs abuse [7–12].

In the case of alcoholic drinks, ethanol is extensively metabolized in the liver via an oxidation process to acetaldehyde and acetic acid [13]. However, another very small fraction of the ethanol dose undergoes a conjugation reaction with glucuronic acid and sulfate, to produce ethyl glucuronide (EtG) and ethyl sulfate (EtS), respectively, which are excreted in the urine (median values of 0.020% as EtG and 0.011% as EtS, on molar basis respect to the ethanol ingested) [14,15]. Both substances (EtS and EtG) have been proposed as efficient markers of alcohol intake and abuse [13,16–19]. They can be detected in body fluids (such as urine and blood) for an extended time period, up to some days in urine, after complete elimination of alcohol [14,15]. Thus, a new method based on the

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sewage epidemiology philosophy, i.e. tracing the marker of alcohol ingestion (EtS) in wastewater has been recently proposed by Reid et al. [20]. The other possible marker of alcohol ingestion, EtG, was also detected in wastewater, but it proved to be unstable in this matrix [20].

Most of the procedures developed for the analysis of EtS and EtG residues in biological samples are normally performed by liquid chromatography–mass spectrometry (LC–MS) or liquid chromatography–tandem mass spectrometry (LC–MS/MS) due to the low volatility of these substances [13–19,21–25]. However, the limits of quantification and detection (LODs and LOQs) of those methods are insufficient for the analysis of these metabolites in wastewater. On the other hand, EtS and EtG are poorly retained in conventional reversed-phase (RP) chromatographic columns, requiring highly aqueous conditions for an adequate retention and post-column addition of an organic modifier to achieve adequate MS sensitivity [13–15,17–19,21–25]. Thus, the method published by Reid et al. [20] was based on ion-pair RP-LC–triple-quadrupole–MS/MS (IP-RP-LC–QqQ–MS/MS) by including an ion-pairing agent (dihexylammonium acetate) into the mobile phase.

Besides, the low m/z value of EtS and its product ions may render problems for positive confirmation by LC–QqQ–MS/MS according to the 2002/657/EC regulation [26] on identification points that has become a standard in Europe for environmental analysis when a QqQ system is employed, as already described for other analytes [27,28].

Thus, in this work we present a new method for the direct determination of EtS in wastewater by LC–MS(/MS). Different LC mechanisms were considered in order to increase EtS retention: IP-RP, pure RP and hydrophilic interaction LC (HILIC). Also a QqQ instrument and a quadrupole time-of-flight (QTOF) system were compared in its quantitative and qualitative performance. Finally, the developed method was applied to wastewater samples in order to evaluate alcohol consumption in a Galician (NW Spain) city along a week.

2. Materials and methods

2.1. Chemicals and stock solutions

Sodium salts of EtS and ethyl-d5 sulfate (EtS-d5) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All concentrations along the manuscript refer, however, to the free neutral EtS and EtS-d5 species. Individual stock solutions were prepared in acetonitrile (Merck, Darmstadt, Germany) and diluted in methanol (Romil, Barcelona, Spain) as necessary. Ultrapure water was obtained in the lab from a Milli-Q water generator (Millipore, Billerica, MA, USA). Ammonium acetate was from Fluka (Steinheim, Germany), tetrabutylammonium bromide (TBA, 99% purity) and formic acid were from Sigma–Aldrich (Steinheim, Germany).

2.2. Samples

Wastewater samples were collected in the course of a week in April 2012 from a sewage treatment plant (STP) serving an urban population of approximately 100,000 inhabitants of the northwest of Spain (Galicia). The identity of the city cannot be disclosed ought to the confidentiality agreement. An automatic sampler (Liquiport 2000, Endress Hauser Weil am Rhein, Germany) working in a time-proportional mode (150 mL collected every 10 min during 24 h) was used to collect representative composite samples of raw wastewater. Sampling was initiated a Tuesday at 9 AM and the first sample was collected after 24 h, initiating sampling of the subsequent day.

Within 3 days after sampling, the samples were vacuum filtered, first through glass fiber prefilters and subsequently through 0.22 μm nitrocellulose filters (Millipore, Bedford, MA). Then, the deuterated internal standard (IS) EtS-d5 at 10 $\mu g\,L^{-1}$ level and TBA at a concentration of 50 mM were added to 1.5 mL of sample. EtS was measured by LC–MS(/MS) as in Section 2.5.

2.3. Sample stability assessment

Stability experiments were performed in closed 16 mL amber vials maintained at room temperature (20 ± 2 °C), refrigerated (4 ± 1 °C) or frozen (−20 ± 2 °C). These studies were done with 10 mL of unfiltered influent wastewater spiked with 50 $\mu g\,L^{-1}$ of EtS (three replicates for each temperature). Several 1 mL aliquots of each solution were taken at different times: between 0 and 1 week (20 °C and 4 °C samples) and between 0 and 4 weeks (−20 °C samples). Each aliquot was filtered through 0.22 μm syringe filters (13 mm GHP membrane, Millipore, Bedford, MA, USA) and subsequently 10 $\mu g\,L^{-1}$ of EtS-d5 and 50 mM TBA was added. Finally, 100 μL of each aliquot was analyzed by LC–QTOF–MS.

2.4. LC–QqQ–MS/MS

The liquid chromatographic system used is equipped with two ProStar 212 high-pressure mixing pumps (Varian, Walnut Creek, CA, USA), a Metachem Technologies vacuum membrane degasser (Bath, UK), an autosampler and a thermostated column compartment ProStar 410 module (Varian). Two silica-based columns were used: a 100 mm × 2 mm Luna 3 μm HILIC column (Phenomenex, Torrance, CA, USA) and a 100 mm × 2.0 mm Synergi 4 μm Fusion-RP column (Phenomenex). Different eluents were considered as detailed in Section 3.1.

Under the optimal conditions, the determination of EtS was carried out on the Synergi Fusion-RP column at a flow rate of 0.4 mL min^{-1} and 45 °C. Eluent A consisted of Milli-Q water and B of MeOH, both containing 0.1% formic acid. Gradient was as follows: 0 min, 0% B; 3 min, 0% B; 5 min, 100% B; 8 min, 100% B; 8.01 min, 0% B; 10 min, 0% B. The injection volume was also optimized and finally set to 100 μL. A post-column switching valve was used to divert the eluent to waste from 0 to 5 min and from 6.5 to 10 min.

The system was interfaced to a Varian 320-MS triple quadrupole mass spectrometer equipped with an electrospray (ESI) interface. Nitrogen was provided by a nitrogen generator (Domnick Hunter, Durham, UK) and used as nebulizing and drying gas. Argon (99.999%) was used as collision gas. Electrospray parameters were as follows: nebulizing gas: 55 psi, 50 °C; drying gas: 18 psi, 200 °C; ion spray voltage: −4500 V.

EtS and EtS-d5 were determined in the electrospray negative mode and multiple reaction monitoring (MRM) mode of acquisition. According to the 2002/657/EC decision [26], two different MS/MS transitions are required to confirm the identity of target analytes. Thus, the most intense MS/MS transition was selected for quantification in the MRM mode and the second one was used for confirmation (Table 1).

2.5. LC–QTOF–MS(/MS)

LC–QTOF–MS analysis was performed on an Agilent 1200 Series (Agilent Technologies, Santa Clara, CA, USA) liquid chromatographic system consisting of a membrane degasser, a binary high-pressure gradient pump, a thermostated LC column compartment and an autosampler. Separation of analytes was performed on the Synergi Fusion RP-column under the same conditions indicated for the LC–QqQ system. However, switching to waste was programmed from 0 to 7 min and from 9 to 10 min, due to the