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Determination of high-intensity sweeteners in river water and wastewater by solid-phase extraction and liquid chromatography–tandem mass spectrometry

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ABSTRACT

High-intensity sweeteners have been suggested as potential organic contaminants due to their widespread use in food, drugs and sanitary products. As a consequence, they are introduced into the environment by different pathways, affecting aquatic life. In this study, a method based on solid-phase extraction (SPE) and liquid chromatography–tandem mass spectrometry (LC–MS–MS) has been developed and validated for the determination of eight sweeteners (saccharin, cyclamate, aspartame, acesulfame, neohesperidin dihydrochalcone, sucralose, stevioside and glycyrrhizic acid) in river water and wastewater. To get the maximum recoveries in SPE, several commercial sorbents were tested and Oasis HLB gave the best results, with recoveries higher than 41% for all of the compounds in the different matrices. Method limits of detection were in the range of 0.001–0.04 μg/L in river water and 0.01–0.5 μg/L in influent and effluent wastewater. Method reproducibility between days ($n = 5$) was below 15% for all compounds. The method was applied to the determination of sweeteners in various river waters and wastewaters in Catalonia. Cyclamate, aspartame, neohesperidin dihydrochalcone, acesulfame and sucralose were found in river water, with the two last compounds being present at the highest values (1.62 μg/L for acesulfame and 3.57 μg/L for sucralose). In influent and effluent wastewater, all of the compounds were found at concentration levels ranging from 0.05 to 155 μg/L except for stevioside and neohesperidin dihydrochalcone, which were not detected.

1. Introduction

Sweeteners are defined as food additives that are used or intended to be used either to impart a sweet taste to food or as a tabletop sweetener. High-intensity sweeteners (HIS) are mainly used as a treatment for obesity, body weight maintenance, management of diabetes and prevention and reduction of dental caries [1,2]. Currently, in the European Union, HIS such as acesulfame, aspartame, cyclamate, neohesperidin dihydrochalcone, saccharin, sucralose and steviol glycosides (regulated since 2010) are permitted as food additives in foodstuffs [3–5].

Most HIS and their metabolites are rapidly absorbed in the gastrointestinal tract. For instance, acesulfame and saccharin are not metabolized and are excreted unchanged by the kidney. Sucralose, stevioside and cyclamate undergo degrees of metabolism, and their metabolites are also excreted [6]. Studies indicated the existence of 2 hydrolysis products of sucralose, 4 CG and 1,6 DCF [7]. Oral stevioside was completely degraded into steviol in pigs [8] while cyclamate was metabolized up to 60% to cyclohexylamine in the human body [9]. After being excreted by the human body and/or discharged from households and industries, HIS reach sewage treatment plants (STPs), but they may resist conventional wastewater treatment processes because of their extreme stability under biological, physical and chemical exposure. Consequently, they are discharged into ground and river waters [10].

Reviews on the issue have highlighted the widespread distribution of HIS in different aquatic environmental matrices [11–13], with levels of acesulfame and sucralose being reported as the highest (up to hundreds of μg/L) [14–16]. The ecotoxicological impact of the presence of several HIS in the environment has been the focus of study in aquatic and terrestrial organisms, such as saccharine exposure in zebrafish embryos [17], and stevioside and aspartame exposure or dietary administration in rodents and salmonella [8,18]. Likewise, the toxicity of acesulfame, cyclamate, saccharine and sucralose in species such as the algae *Scenedesmus vacuolatus*, water fleas *Daphnia magna* and duckweed *Lemna minor* [10,19] and in aquatic plants [20] has been evaluated, but all of the above experiments showed low hazard

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and risk potential towards these organisms. Nonetheless, other studies have concluded that HIS could produce potentially harmful effects on ecosystems and human health. Wiklund et al. [21] investigated the behavioural and physiological effects of sucralose on two crustaceans and their results showed alteration in swimming height, increased swimming speed and the time taken to reach food and shelter was prolonged. Reliable analytical methodologies are required to quantify low levels of HIS in a broad range of environmental matrices. Solid-phase extraction (SPE) (off-line SPE [16,22] and on-line SPE [23–25]) have been used to extract these compounds from environmental water samples. For these purposes, the copolymer sorbents Oasis HLB (lipophilic divinylbenzene-hydrophilic N-vinylpyrrolidone) [26–28], Isolute ENV+ (hydroxylated polystyrene-divinylbenzene (PS-DVB)) [29], HR-X (hydrophobic PS-DVB) [14], Bakerbond SDB-1 (styrene-divinylbenzene (SDVB)) [30] and PWAX (polymer weak anion exchange) [31] have been used to extract HIS from complex aqueous samples. Moreover, two SPE cartridges in tandem, Bond Elut PPL (SDVB) and Bond Elut NH_2 (weak anion exchange), were used for extracting and additional clean-up [32], but this method was only developed for sucralose.

Meanwhile, some studies avoided the use of any extraction technique. Samples were only filtered and then directly injected into the chromatographic system. However, higher detection limits (5 μg/L) were reported in those studies [19,20].

With regard to chromatographic techniques, gas chromatography [33], reversed-phase liquid chromatography [15,34–36], ion chromatography [19,37] and ion-pair chromatography [31] have been used to determine these compounds in several environmental matrices. Most of the published methods use reversed-phase columns such as C_{18} [14,24,28,31] and C_8 [27,29,30], although other LC retention mechanisms for these polar compounds have been considered, such as hydrophilic interaction (HILIC) [22]. With respect to mass spectrometry, triple quadrupole (QqQ) has widely been applied in quantitative trace analysis of HIS, hence the sensitivity and specificity of the analytical methods is increased [24–26,29]. Additionally, time of flight mass spectrometry has also been used in a previous study [27] in which aspartame, saccharine and sucralose were determined in different environmental water and beverage samples.

The objective of this work is the development and validation of a simple, reliable and sensitive analytical method for the simultaneous determination of eight HIS (acesulfame, saccharin, cyclamate, aspartame, sucralose, stevioside, neohesperidin dihydrochalcone and glycyrrhizic acid) in river water and wastewater, using SPE and liquid chromatographic–(electrospray) tandem mass spectrometry (LC–(ESI)MS/MS). It should also be highlighted that a polar-embedded reversed-phase column with fused-core particles was used and several strategies for removing or reducing the matrix effect were studied. To the best of our knowledge, this is the first study where determination of stevioside and glycyrrhizic acid in environmental water samples is studied.

2. Experimental

2.1. Reagents and standard

Acesulfame-K (ACE) (6-methyl-1,2,3-oxathiazin-4(3H)-one-2,2-dioxide, potassium salt), saccharin-Na (SAC) (1,2-benzisothiazol-3(2H)-one-1,1-dioxide, sodium salt), cyclamate-Na (CYC) (Cyclohexylsulfamic acid, sodium salt), aspartame (ASP) (N-L-α-aspartyl-L-phenylalanine-1-methyl ester), sucralose (SUC) (1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl 4-chloro-4-deoxy-α-D-galactopyranoside), stevioside (STV) (13-[(2-O-β-D-glucopyranosyl-α-D-glucopyranosyl)oxy]-kaur-16-en-18-oic acid-4 α-β-D-glucopyranosyl ester), neohesperidin dihydrochalcone (NHDC) (3,5-dihydroxy-4-(3-hydroxy-4-methoxy-hydrocinnamoyl) phenyl-2-O-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside) and glycyrrhizin acid (GLY) (α-D-Glucopyranosiduronic acid,(3 β,20 β)-20-carboxy-11-oxo-30-norolean-12-en-3-yl 2-O-β-D-glucopyranuronosyl) were purchased from Sigma–Aldrich (St. Louis, USA). All standards had a purity higher than 96% except for GLY (70%). Deuterated compounds sucralose-d_6 and aspartame-d_3 were purchased from LGC Standards (Wesel, Germany), with an isotopic purity of 98% and were used as surrogates.

Stock solutions of individual standards and deuterated standards were prepared by dissolution of pure compound in methanol at a concentration of 1000 mg/L and 100 mg/L of the neutral species, respectively, and then stored at −20 °C in amber glass bottles. Mixed intermediate standard solutions were prepared every month by dilution of stock solutions in MeOH at a concentration of 2 mg/L and stored at 4 °C. Mixed standard working solutions were prepared daily from intermediate standard solutions by appropriate dilution with water:MeOH (9:1, v/v).

Acetone, acetonitrile (ACN), dichloromethane (DCM), isopropyl alcohol (IPA), ethyl acetate (EtOAc), hexane, methanol (MeOH) and methyl tert-butyl ether (MTBE) were purchased from Prolabo (VWR, Llinars del Vallès, Spain). All reagents were HPLC grade. Formic acid (HCOOH) for LC–MS analysis was purchased from Merck (Darmstadt, Germany) and nitrogen gas was sourced from Carburos Metálicos (Tarragona, Spain). Ultrapure water was obtained using an ultrapure water purification system provided by Veolia Water (Sant Cugat del Vallès, Spain). Acetic acid (CH_3COOH), ammonium acetate (CH_3COONH_4), ammonium formiate ($HCOONH_4$), ammonium hydroxide (NH_4OH), Tris (hydroxymethyl) amino methano (TRIS) and hydrochloric acid (HCl), were purchased from Sigma–Aldrich.

2.2. Water sampling and sample pre-treatment

Grab samples of influent and effluent wastewater were collected over five months from two sewage treatment plants (STPs) located in the area of Tarragona. Each STP receives between 18,000 and 24,000 m^3/day of urban wastewater and some industrial discharges. Wastewater treatment includes physical, chemical and biological processes. River water was collected from five rivers (Francolí, Ter, Llobregat, Segre and Ebre) located in Catalonia (Spain). These rivers empty into the Mediterranean Sea, except for the Segre river that empties into the Ebro river. The waters of these rivers are extensively used for household, industrial, irrigation and livestock uses. The river waters were sampled at a depth of approximately 1.0 m below the surface.

All samples were collected using pre-cleaned polyethylene bottles, acidified to pH 3 with HCl and immediately stored until analysis at −20 °C. Prior to SPE, samples were filtered using a 1.2 μm glass fibre filter (Fisherbrand, Loughborough, UK), except in the case of the influent sample, which was first centrifuged (Hettich Zentrifugen, Tuttlingen, Germany) at 9000 rpm for 7 min before being filtered.

2.3. Sample extraction by SPE

The following SPE commercial cartridges were tested: Oasis HLB (500 mg) and Oasis MAX (150 mg) from Waters (Wexford, Ireland), Discovery DPA-6S (500 mg), Discovery DSC-Diol (500 mg) and Supelclean LC-SAX (200 mg) from Supelco (Bellefonte, USA), Bond Elut Plexa (200 mg) and Bond Elut SCX (200 mg) from Agilent (Santa Clara, USA). 6 cc SPE cartridges lab-packed with 500 mg of Florisil (bulk adsorbent from Sigma–Aldrich) were also used.