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Artificial sweeteners in beverages by ultra performance liquid chromatography with photodiode array and liquid chromatography tandem mass spectrometry

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ABSTRACT

Two fast, accurate and sensitive liquid chromatography methods have been developed and optimized for a better control of the content of artificial sweeteners in industrial beverages. Ultra performance liquid chromatography coupled with photodiode array (UPLC–PDA) and liquid chromatography-electrospray ionization-tandem mass spectrometry (LC–ESI–MS/MS) methods were implemented for the monitoring of aspartame, neohesperidine dihydrochalcone, neotame, potassium acesulfame, saccharin, sodium cyclamate and sucralose in beverages marketed as "sugar-free" or "diet," including soft and powdered drinks. Minimal sample preparation procedure consisting on a simple dilution and filtration is required before analysis. The methods showed excellent linearity ($R^2 < 0.9990$) for target compounds. Limits of quantification (LOQs) were far below the legal requirements for all considered compounds (0.01–0.1 μg mL^{-1} and 0.05–5 ng mL^{-1} for UPLC–PDA and LC–MS/MS, respectively). Precision and recovery studies in real samples showed excellent results. The recoveries at two concentration levels ranged between 90.0 and 114.6%, with relative standard deviations lower than 9.4 RSD%. Finally, the proposed methodology was successfully applied to the analysis of artificial sweeteners in 66 beverage products commonly consumed in Spain. Different sample categories were evaluated, including energy drinks, soft drinks, juices, teas, soy beverages, dairy-based drinks, beers, and spirit alcoholic drink, and proved its suitability for quick and reliable application in quality control laboratories.

1. Introduction

Artificial sweeteners are an important class of additives, commonly used in food and beverage industries and regulated by the Food and Drug Administration (FDA) and European Union (EU). Since recently they are also considered as emerging environmental contaminants due to their presence in wastewater (Kokotou, Asimakopoulos, & Thomaidis, 2012; Lange, Scheurer, & Brauch, 2012). Moreover, although they have been considered as safe by the European Food Safety Authority (EFSA) (EFSA, 2009; Kroger, Meister, & Kava, 2006; Nofre & Tinti, 2000; Scientific Committee on Food (SCF), 1985; Serra-Majem et al., 2003; Shankar, Ahuja, & Sriram, 2013; Tandel, 2011), concerns about health risks have arised (Kroger et al., 2006; Mortensen, 2006; Soffritti, Belpoggi, Tibaldi, Esposti, & Lauriola, 2007; Tandel, 2011). Current legislation limits the content of food additives in foodstuffs. Seven artificial sweeteners including acesulfame (E 950), aspartame (E 951), cyclamic acid and its salts (sodium cyclamate, E 952), saccharin and its salts (E 954), sucralose (E 955), neohesperidine dihydrochalcone (E 959) and neotame (E 961), are authorized in European Union (EU), directive 94/35/EC (European Commission, Directive 94/35, 1994). with four amendments (European Commission, Directive 96/83, 1997; European Commission, Directive 2003/115, 2004; European Commission, Directive 2006/52, 2006; European Commission, Directive 2009/163, 2009) for use in modern food industry. In the USA artificial sweeteners are part of the Generally Recognized as Safe (GRAS) ingredients (GRAS, 2013a), but the corresponding list does not include cyclamates (banned in USA) (GRAS, 2013b) and neohesperidine dihydrochalcone. Since sweeteners are mostly used in combination each other, fast, simple, sensitive and high throughput analytical methodologies are required to measure levels of sweeteners in a broad range of food matrices.

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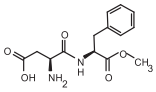
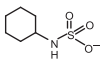
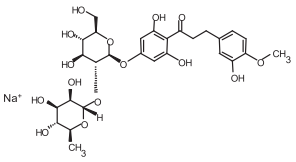
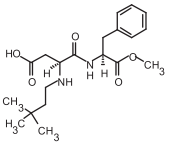
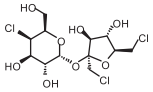
0956-7135/

Table 1
Analyte structures and other relevant data of the studied compounds.

	ACE	ASP	CYC	NHDC	NEO	SAC	SUC
Structure[a]							
CAS N°[a]	55589-62-3	22839-47-0	139-05-9	20702-77-6	165450-17-9	81-07-2	56038-13-2
Formulae[a]	$C_4H_4KNO_4S$	$C_{14}H_{18}N_2O_5$	$C_6H_{12}NO_3SNa$	$C_{26}H_{36}O_{15}$	$C_{20}H_{30}N_2O_5$	$C_7H_5NO_3S$	$C_{12}H_{19}Cl_3O_8$
Molecular weight[a]	201.24	294.30	201.22	612.58	378.46	183.18	397.63
pKa[a]	~2	3.71	−8.66[c]	6.85	3.68	1.60	12.52
log Kow[a]	−0.31	0.542	−2.63	0.205	3.834	0.910	0.229
Water solubility (g L^{-1})[b]	270	10	1000	0.4–0.5	12.6	4	110
E-N°	E-950	E-951	E-952	E-959	E-961	E-954	E-955
Maximum usable dose (mg L^{-1})[d]	350	600	250	30	20	80[e]	300

[a] Data from SciFinder Scholar Database (Calculated using Advanced Chemistry Development (ACD/Labs) Software VII. 02 (©1994–2011 ACD/Labs)): http://www.cas.org/products/sfacad/.
[b] Experimental values, from database of physicochemical properties. Syracuse Research Corporation: http://www.syrres.com/esc/physdemo.htm.
[c] Protonated form.
[d] Maximum usable dose (MUD) authorized in EU legislation for use in non-alcoholic drinks. European Commission, Directive 94/35, 1994; European Commission, Directive 96/83, 1997; European Commission, Directive 2003/115, 2004; European Commission, Directive 2006/52, 2006 and European Commission, Directive 2009/163, 2009).
[e] 'Gaseosa': non-alcoholic water based drink with added carbon dioxide, sweeteners and flavourings, 100 mg L^{-1}.

The methodologies for the determination of artificial sweeteners in food, drinks and dietary products have been recently reviewed by Zygler, Wasik, and Namieśnik (2009). Beverages samples characterized by relatively simple matrix can be diluted or dissolved in deionized water or an appropriate buffer. In the case of carbonated drinks, the samples are degassed prior to analysis (Demiralay, Özkan, & Guzel-Seydim, 2006; Herrmannová, Křivánková, Bartoš, & Vytřas, 2006; Zhu, Guo, Ye, & James, 2005) and solid-phase extraction can be employed to eliminate interferences (McCourt, Stroka, & Anklam, 2005; Wasik, McCourt, & Buchgraber, 2007; Zygler, Wasik, Kot-Wasik, & Namieśnik, 2011). High performance liquid chromatography (HPLC) (Demiralay et al., 2006; Dossi, Toniolo, Susmel, Pizzariello, & Bontempelli, 2006; George, Arora, Wadhwa, & Singh, 2010; Wasik et al., 2007) is the most widely used technique, although other separation methods such as ion chromatography (IC) (Chen et al., 2001; Zhu et al., 2005), thin-layer chromatography (TLC) (Idris, Srivastava, Baggi, Shukla, & Ganjoo, 2010; Morlock et al., 2007), capillary electrophoresis (CE) (Herrmannová et al., 2006; McCourt et al., 2005; Stojkovic, Mai, & Hauser, 2013) and gas chromatography (GC) (Hashemi, Habibi, & Jahanshahi, 2011) have also been shown useful to analyse food additives. Various detection systems, including ultraviolet (UV) (Demiralay et al., 2006; Dossi et al., 2006; George et al., 2010), mass spectrometry (MS) (Ferrer & Thurman, 2010; Scheurer, Brauch, & Lange, 2009; Zygler et al., 2011), and evaporative light-scattering (ELSD) (Wasik et al., 2007) have been coupled with these techniques. UV detection mode is not suitable for determination of sodium cyclamate and sucralose, because of the lack of UV chromophore in the molecule, and consequently a previous derivatization procedure is needed (Idris et al., 2010; Morlock & Prabha, 2007). For this and other reasons few UPLC methods for the concurrent determination of these sweeteners exist and usually have been based on detection by mass-spectrometry (Zygler et al., 2009).

The aim of the present study was to optimize and validate two methods for the determination of artificial sweeteners in beverage samples. Acesulfame (ACE), aspartame (ASP), neohesperidine dihydrochalcone (NHDC), neotame (NEO) and saccharin (SAC) were analysed by means of ultra performance liquid chromatography coupled with photodiode array (UPLC–PDA), while these sweeteners together with sodium cyclamate (CYC) and sucralose (SUC) were analysed using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS). Compounds structures and other relevant data are shown in Table 1. The proposed methodology was applied using two different instrumental systems (PDA and MS/MS detection) as well as two different LC columns, with a minimal sample treatment. Sixty six beverages were analysed to evaluate the foods safety with respect to the maximum usable dose (MUD) of sweeteners in foodstuffs in accordance with the European Union legislation.

2. Material and methods

2.1. Chemicals and standards

Saccharin (≥99%), sucralose (≥98%), neohesperidine dihydrochalcone (≥95%), neotame (≥98%) and potassium acesulfame (≥99%) were from Sigma Aldrich (Madrid, Spain). Sodium cyclamate and aspartame were purchased from Supelco (Bellefonte PA, USA) and sucralose-d_6 (96%) was obtained from Toronto Research Chemicals (Toronto, Ontario, Canada). Stock solutions of each individual compound were prepared at 2 mg mL^{-1} in methanol. Diluted standard mixtures used for spiking beverage samples were prepared in methanol to appropriate concentration levels, whereas diluted standard mixtures used as calibration solutions were

Table 2
Optimized LC–ESI–MS/MS conditions for selected compounds.

Analyte	t_r (min)	MRM 1 (quantification)	Cone voltage (V)	CE (eV)	MRM 2 (confirmation)	CE (eV)
ACE	6.38	161.9 > 82	−45	−20	161.9 > 78	−40
SAC	11.82	181.9 > 42	−70	−48	181.9 > 106	−26
CYC	13.96	177.9 > 79.9	−85	−36	177.9 > 81	−28
SUC-d6	17.62	400.95 > 364.9	−85	−18	400.95 > 35.1	−34
SUC	17.71	395 > 358.8	−80	−16	397 > 361	−16
ASP	19.72	293 > 261	−80	−14	293 > 200	−20
NHDC	23.12	611.2 > 303	−130	−50	611.2 > 125	−56
NEO	27.28	377.2 > 199.9	−90	−24	377.2 > 345	−18

t_r – Retention time; CE – Collision energy.