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Retention behaviour of some high-intensity sweeteners on different SPE sorbents

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ABSTRACT

The objective of this paper is to provide information about application of solid-phase extraction (SPE) for isolation of nine high-intensity sweeteners (acesulfame-K, alitame, aspartame, cyclamate, dulcin, neotame, saccharin, sucralose and neohesperidin dihydrochalcone) from aqueous solutions. The influence of several types of LC–MS compatible buffers (different pH values and compositions) on their recovery has been studied and discussed. A number of commercially available SPE cartridges, such as Chromabond C18ec, Strata-X RP, Bakerbond Octadecyl, Bakerbond SDB-1, Bakerbond SPE Phenyl, Oasis HLB, LiChrolut RP-18, Supelclean LC-18, Discovery DSC-18 and Zorbax C18 were tested in order to evaluate their applicability for the isolation of analytes. Very high recoveries (better than 92%) of all studied compounds were obtained using formic acid–N,N-diisopropylethylamine buffer adjusted to pH 4.5 and C_{18}-bonded silica sorbents. Behaviour of polymeric sorbents strongly depends on their structure. Strata-X RP behaves much like a C_{18}-bonded silica sorbent. Recoveries obtained using Oasis HLB were comparable with those observed for silica-based sorbents. The only compound less efficiently (83%) retained by this sorbent was cyclamate. Bakerbond SDB-1 shows unusual selectivity towards aspartame and alitame. Recoveries of these two sweeteners were very low (26 and 42%, respectively). It was also found that aspartame and alitame can be selectively separated from the mixture of sweeteners using formic acid–triethylamine buffer at pH 3.5.

1. Introduction

Sweetness is one of the most pleasurable sensations for human beings. For ages, natural carbohydrates or their mixtures were used to impart sweet taste on foods. Besides making foods pleasurable to eat, carbohydrates provide a significant amount of energy to the body. Today we are aware of the fact that excess sugar consumption is of a significant concern, due to its correlation with a number of adverse health related issues (*e.g.* obesity, dental problems, diabetes control). High-intensity sweeteners are valuable alternatives to sugar, they provide sweetness sensation while no or very little energy is produced during their metabolism. Moreover, they do not promote growth of dental flora nor raise blood glucose levels [1]. In this regard, high-intensity sweeteners are perfect sugar substitutes for those who want/have to control their sugar consumption for various reasons.

The class of high-potency sweeteners includes three categories: synthetic, semi-synthetic and natural sweeteners. Nine substances are within the scope of this article. Acesulfame-K (ASC-K), alitame (ALI), aspartame (ASP), cyclamate (CYC), dulcin (DUL), neotame (NEO) and saccharin (SAC) are synthetic sweeteners. Sucralose (SCL) and neohesperidin dihydrochalcone (NHDC) are semi-synthetic sweeteners.

The use and consumption of artificial sweeteners is increasing. Today, a consumer can enjoy a wide range of low-calorie foods, starting from drinks and chewing gums to cakes and chocolates [2]. It should be noted that there is a common trend within the food industry to use sweetener blends. Use of sweetener blends has some important advantages, *i.e.* it allows to decrease the total amount of sweeteners needed (due to synergistic effect) and improves the overall taste profile of a final product [3].

In most countries, sweeteners, just like any other food additives, need to be authorised before they can be used for food production. This is to ensure consumer's safety. During the authorisation process, maximum usable dose (MUD) of a sweetener is determined. Some sweeteners are authorised to be used at *quantum satis* level. It means that no maximum level is specified. However, sweeteners shall be used in accordance with good manufacturing practice, at a dose level not higher than is necessary to achieve the intended purpose and provided the consumer is not misled [4]. The MUD value is calculated taking into account the average consumption level of a given type of a foodstuff and its acceptable daily intake (ADI), which is the amount of a substance that can be ingested over a lifetime without an appreciable health risk, plus some error margin.

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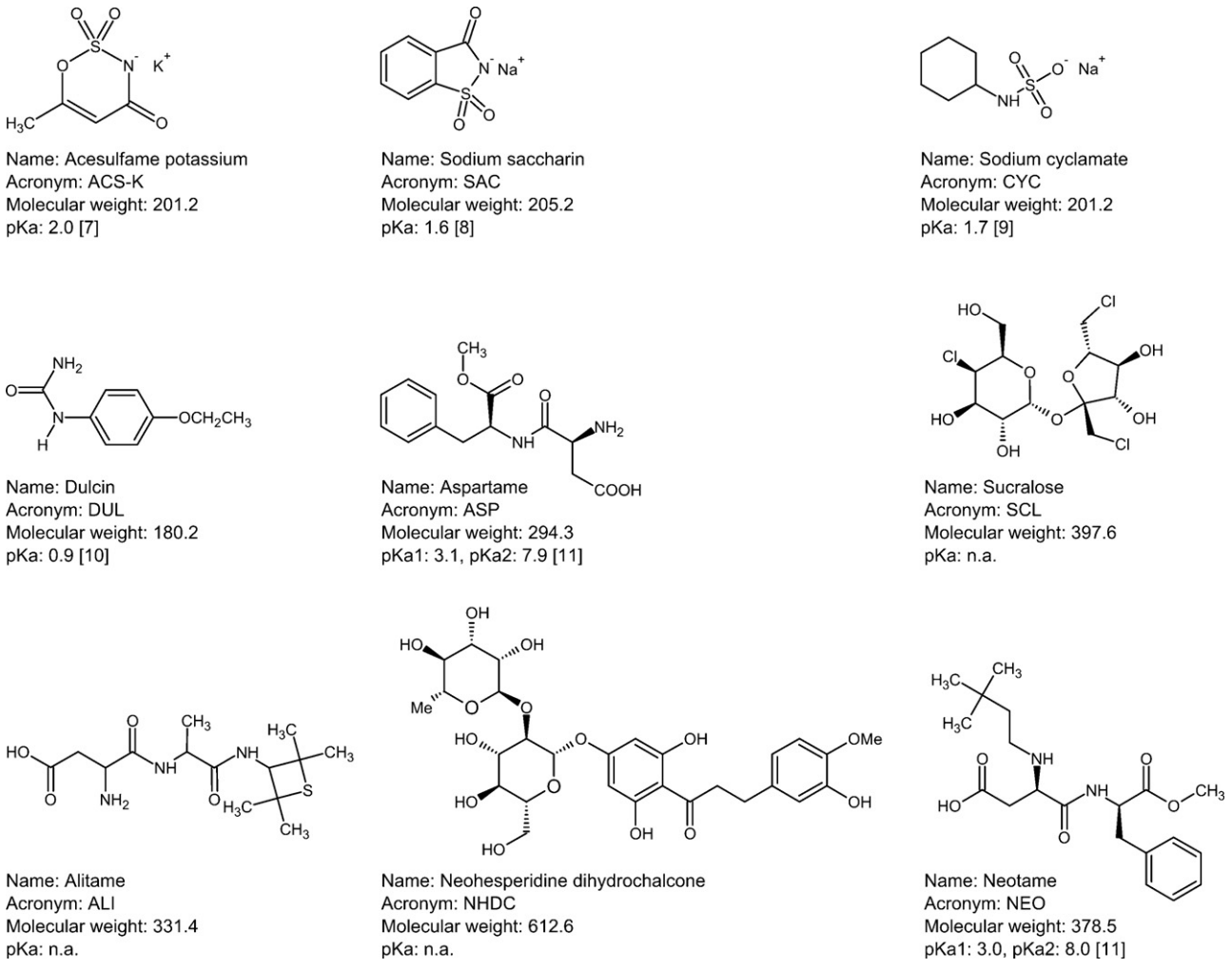

Fig. 1. Structures of sweeteners under the study.

For health reasons it is of utmost importance to have control over the amount of sweeteners (and other additives) used for food production. This can only be achieved when appropriate legislation and tools to enforce this legislation are available.

While in most cases the legislation is in place, there is a lack of analytical methods capable to provide reliable data concerning multiple sweeteners being in use today. Existing official methods of analysis cover just a few of many sweeteners being authorised presently. Therefore an accent must be put on the development of analytical methods capable to deliver reliable, quantitative and qualitative information concerning composition of different foodstuffs.

Foods cover a broad range of physical and chemical forms, from dry powders and liquids containing just a few ingredients to multicomponent, complex mixtures containing lipids, proteins, colorants, thickening agents, sweeteners, natural extracts and preservatives.

Sample preparation technique depends on the sample matrix and the properties and level of the sweeteners to be determined [5]. The final extract of most sample preparation procedures is an aqueous solution containing the compounds. Such a solution usually requires an additional clean-up step before final determination. In case of analysis of artificial sweeteners, solid-phase extraction (SPE) is the most frequently used technique for this purpose. Indeed, it offers better versatility and selectivity than the other extraction techniques. SPE is recognized as beneficial alternative to liquid–liquid extraction, because it overcomes many drawbacks of LLE [6]. It provides lower intrinsic costs, reduction of processing time, low solvent consumption, prevention of possible emulsion formation and the possibility of automated processing.

Today, an analytical chemist can choose from a large number of different SPE products available on the market. The selection of an SPE sorbent is crucial for obtaining good results [7], however, it is not an easy task. Detailed specifications of sorbents are not always available, multiple types of analyte–sorbent interactions and other effects (*e.g.* ion-pair formation) make theoretical prediction of a retention behaviour of a compound or group of compounds, on a given SPE sorbent, very difficult – if possible at all. This is particularly true in case of high-intensity sweeteners belonging to different classes of chemicals (peptides, sulfamates, glycosides and carbohydrate derivatives) and covering a wide range of polarities (Fig. 1).

The aim of this paper is to investigate the applicability of various SPE sorbents for isolation of nine intense sweeteners. Octadecyl modified silica sorbents were successfully applied for isolation of artificial sweeteners [13,18–20]. Several other silica-based and polymeric SPE sorbents were tested under the conditions given in these papers. Additionally, the impact of the composition and pH value of buffers being used, on the recovery of analytes were also studied. The results presented below were obtained within the framework of development of an HPLC–MS method for determination of artificial sweeteners in various food products.

2. Materials and methods

2.1. Reagents and materials

HPLC gradient grade methanol was purchased from Merck (Darmstadt, Germany). Acetone (pure p.a.), formic acid (pure p.a.), ammonia (pure p.a.) were obtained from P.O.Ch. (Gliwice, Poland), acetic acid (pure p.a.) from Merck (Darmstadt, Germany) and triethylamine (pure p.a.) from Fluka (Belgium). N,N-diisopropylethylamine (DIPEA) was purchased from Sigma–Aldrich. Ultrapure water was obtained from an HLP5 system (Hydrolab, Poland).

The individual standards of studied artificial sweeteners were obtained from different sources: ACS-K from Nutrinova (Frankfurt am Main, Germany), ALI from Frapp's Pharma (Hong-Kong, China), ASP from Ajinomoto (Switzerland), CYC from Merck (Germany), NEO from CHEMOS (Regenstauf, Germany), NHDC from Sigma–Aldrich (Germany), SCL from Nestlé (Obre, Switzerland), SAC from Sigma–Aldrich (Germany), DUL and internal standard (IS)-sodium N-(2-methylcyclohexyl) sulfamate were prepared according to Refs. [14,15].

The following SPE disposable cartridges were tested: Chromabond® C18ec 6 mL/1000 mg and Chromabond® C18ec 6 mL/500 mg (Macherey-Nagel, Germany), Strata-X 33 μm Polymeric RP 3 mL/200 mg (Phenomenex, Germany), Bakerbond Octadecyl 3 mL/200 mg, Bakerbond SDB-1 3 mL/200 mg, Bakerbond Phenyl 3 mL/500 mg (J.T. Baker, The Netherlands), Oasis HLB 3 mL/60 mg (Waters, USA), LiChrolut RP-18 3 mL/500 mg (Merck, Germany), Supelclean LC-18 3 mL/500 mg, Discovery DSC-18 3 mL/500 mg (Supelco, USA) and Zorbax C18 3 mL/200 mg (Agilent Technologies, USA).

2.2. Buffers and standard solutions

Formate buffer (mobile phase component, pH 4.5) was prepared by dissolving 1.5 mL (40 mM) of formic acid in 2 L of water and adjusting the pH to 4.5 with aqueous ammonia solution. The buffer solution was filtered through a 0.45 μm cellulose membrane filter. The HPLC mobile phase components were prepared by mixing methanol with buffer solution and acetone (component A: 69 + 24 + 7, component B: 11 + 82 + 7, v/v/v). The composition of mobile phase was chosen according to previously described procedure [13], but since the use of triethylamine (TEA) for mass spectrometry is not recommended [16], it has been replaced with ammonia.

Seven different buffer solutions were used during this study. Formic acid–ammonia buffer at pH 3.5 (FA-AM-35), formic acid–ammonia buffer at pH 4.5 (FA-AM-45, mobile phase buffer), formic acid–TEA buffer at pH 3.5 (FA-TEA-35), formic acid–TEA buffer at pH 4.5 (FA-TEA-45), acetic acid–TEA buffer at pH 5.8 (AA-TEA-58), acetic acid–TEA buffer at pH 8.4 (AA-TEA-84) and formic acid–DIPEA buffer at pH 4.5 (FA-DIPEA-45). All buffer solutions were prepared in the same way, by titration of 20 mM solution of an acid with an appropriate amine (or aqueous ammonia) until desired pH was reached.

Stock solutions of individual sweeteners (10 mg/mL) were prepared by dissolution of pure sweeteners in water (ACS-K, SAC, CYC, SCL and ALI) or in methanol–water (1:1) mixture. Stock solution of internal standard (0.5 mg/mL) was prepared in water. Intermediate solution of the mixture of sweeteners (1.0 mg/mL) was prepared in water. Working standards (0.1 mg/mL) were prepared by dilution of the intermediate solution with an appropriate buffer. All standards were stored in the dark at 4 °C.

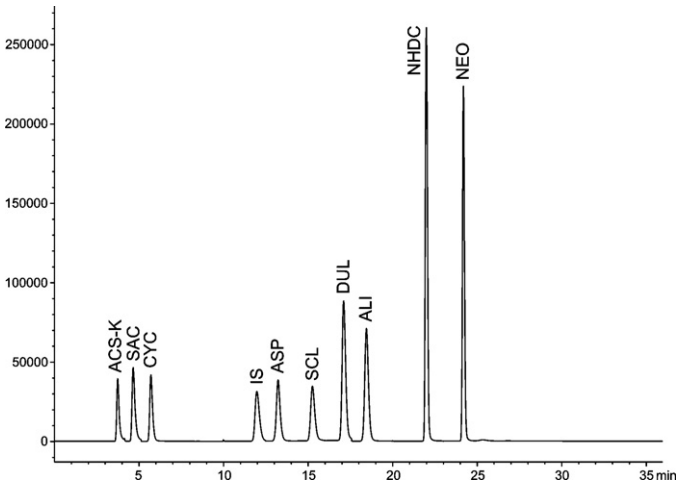

Fig. 2. Chromatographic separation of high-intensity sweeteners. See text for conditions and abbreviations.

2.3. Instrumentation

The chromatographic analyses were performed using an Agilent 1100 series HPLC system. The chromatographic system consisted of G1313A autosampler, with the injection volume set to 20 μL, G1312A binary pump and G1316A thermostated column compartment connected in series with G1313A DAD detector and G1315B MSD mass spectrometer equipped with an electrospray probe. The separation of analytes was performed using Nucleodur C18 Pyramid (250 mm × 3 mm, 5 μm) HPLC column (Macherey-Nagel, Germany). 24-Port vacuum manifold (Grace, USA) was used for solid-phase extractions.

2.4. Solid-phase extraction

A previously described [12,17] SPE procedure was employed for extraction of analytes. Disposable SPE cartridges were conditioned with 3 mL of methanol followed by three 2 mL portions of an appropriate buffer solution. A portion of 2 mL of the working standard was passed through the cartridges at flow rate of approximately 0.5–1 mL/min. After washing the SPE sorbent bed with 3 mL of buffer solution, sweeteners were eluted using two portions of 2 mL of methanol (equilibrating the sorbent bed for 5 min with the first portion of methanol). Before HPLC–MS analysis, the extract containing sweeteners was made up to the volume of 8 mL with mobile phase buffer (FA-AM-45), in order to obtain the solution containing *ca.* 50% of methanol.

Table 1
SIM mode parameters for MS detection.

Analyte	Start time [min]	Fragmentor [eV]	SIM ion [*m*/*z*]
ACS-K	0.00	60	162
SAC	4.15	60	182
CYC	5.15	60	178
IS	10.00	80	192
ASP	12.65	80	293
SCL	14.35	90	511, 395
DUL	16.10	22	225
ALI	17.80	80	330
NHDC	20.00	210	611
NEO	23.00	130	377

Table 2
Recoveries of high-intensity sweeteners obtained using different SPE cartridges and buffers.

Analyte	Buffer	Cartridge type										
		Chromabond C18ec (1000 mg)	Chromabond C18ec (500 mg)	Supelclean LC-18 (500 mg)	Discovery DSC-18 (500 mg)	LiChrolut RP-18 (500 mg)	Bakerbond Octadecyl (200 mg)	Zorbax C18 (200 mg)	Bakerbond Phenyl (500 mg)	Strata-X RP (200 mg)	Oasis HLB (60 mg)	Bakerbond SDB-1 (200 mg)
Recovery % (SD, $n = 3$)												
ACS-K	FA-NH3-35	97.4 (2.2)	36.8 (2.5)	–	–	–	–	–	–	–	–	–
	FA-NH3-45	85.1 (2.3)	31.4 (2.1)	–	–	–	–	–	–	–	–	–
	FA-TEA-35	99.8 (2.1)	96.2 (2.4)	93.5 (3.1)	78.8 (5.2)	96.1 (2.7)	23.2 (7.6)	29.1 (4.5)	73.6 (1.1)	100.7 (0.4)	56.9 (1.8)	99.8 (0.9)
	FA-TEA-45	98.0 (2.2)	99.1 (2.2)	98.5 (2.8)	97.8 (4.1)	97.8 (2.4)	40.4 (5.7)	33.5 (4.2)	95.9 (1.2)	97.9 (0.5)	74.1 (1.8)	97.0 (1.1)
	AA-TEA-58	–	–	–	–	–	47.9 (4.9)	33.7 (4.4)	–	–	82.4 (1.6)	101.8 (1.6)
	AA-TEA-84	–	–	–	–	–	41.7 (5.6)	33.8 (3.9)	–	–	77.4 (2.3)	100.2 (0.9)
	FA-DIPEA-45	–	–	–	–	–	94.2 (3.8)	91.6 (2.7)	–	–	91.6 (1.1)	95.18 (1.7)
SAC	FA-NH3-35	98.8 (2.5)	107.3 (2.7)	–	–	–	–	–	–	–	–	–
	FA-NH3-45	99.8 (2.3)	89.0 (2.4)	–	–	–	–	–	–	–	–	–
	FA-TEA-35	99.4 (2.7)	102.5 (2.6)	94.6 (0.4)	86.9 (4.6)	103.1 (1.2)	25.8 (5.4)	69.5 (3.3)	96.8 (1.5)	98.7 (0.7)	100.1 (0.5)	98.1 (0.9)
	FA-TEA-45	99.0 (2.5)	100.4 (2.4)	101.1 (0.6)	100.6 (3.0)	102.6 (2.9)	96.2 (2.1)	101.1 (3.2)	100.5 (1.8)	103.7 (0.9)	103.7 (0.7)	103.0 (0.8)
	AA-TEA-58	–	–	–	–	–	99.7 (2.2)	95.9 (2.9)	–	–	95.7 (0.8)	99.5 (0.8)
	AA-TEA-84	–	–	–	–	–	102.6 (2.3)	92.9 (3.6)	–	–	103.9 (0.9)	98.1 (0.9)
	FA-DIPEA-45	–	–	–	–	–	100.9 (1.8)	99.6 (2.9)	–	–	97.4 (0.5)	99.6 (1.0)
CYC	FA-NH3-35	97.8 (0.9)	88.4 (1.4)	–	–	–	–	–	–	–	–	–
	FA-NH3-45	97.8 (1.1)	79.3 (1.6)	–	–	–	–	–	–	–	–	–
	FA-TEA-35	99.5 (0.7)	94.4 (1.2)	92.5 (0.3)	84.7 (3.5)	95.0 (1.8)	23.4 (6.2)	41.3 (2.5)	75.8 (1.4)	97.8 (0.6)	30.0 (3.6)	97.1 (0.4)
	FA-TEA-45	97.4 (0.8)	96.8 (1.4)	96.5 (0.5)	95.6 (2.6)	96.3 (1.4)	61.7 (3.1)	80.2 (2.8)	95.5 (1.5)	94.9 (0.7)	41.3 (2.2)	94.6 (1.5)
	AA-TEA-58	–	–	–	–	–	76.9 (3.2)	78.1 (2.1)	–	–	49.5 (1.9)	95.8 (1.2)
	AA-TEA-84	–	–	–	–	–	77.8 (2.8)	71.2 (2.9)	–	–	42.9 (2.6)	92.2 (1.6)
	FA-DIPEA-45	–	–	–	–	–	96.7 (2.6)	96.4 (2.2)	–	–	83.2 (2.4)	96.7 (0.6)
ASP	FA-NH3-35	103.7 (1.5)	108.9 (1.7)	–	–	–	–	–	–	–	–	–
	FA-NH3-45	90.9 (1.3)	91.1 (1.4)	–	–	–	–	–	–	–	–	–
	FA-TEA-35	99.7 (1.4)	95.8 (1.5)	101.9 (1.0)	94.6 (1.4)	95.6 (0.8)	46.4 (3.5)	105.3 (1.8)	99.9 (0.6)	100.8 (1.2)	101.6 (0.3)	0.0
	FA-TEA-45	100.1 (1.2)	100.3 (1.3)	99.2 (0.9)	98.9 (1.2)	97.8 (2.1)	98.4 (2.1)	97.3 (1.9)	96.9 (0.7)	94.9 (1.2)	96.0 (0.4)	0.0
	AA-TEA-58	–	–	–	–	–	102.3 (2.2)	98.8 (1.5)	–	–	98.9 (0.5)	16.32 (1.6)
	AA-TEA-84	–	–	–	–	–	94.8 (2.6)	94.8 (2.1)	–	–	95.6 (0.9)	25.6 (1.3)
	FA-DIPEA-45	–	–	–	–	–	101.5 (2.1)	101.4 (1.6)	–	–	99.4 (0.3)	34.3 (1.4)
SCL	FA-NH3-35	105.5 (2.2)	103.4 (2.1)	–	–	–	–	–	–	–	–	–
	FA-NH3-45	88.6 (2.5)	96.4 (1.9)	–	–	–	–	–	–	–	–	–
	FA-TEA-35	102.6 (2.8)	99.6 (3.2)	103.4 (0.6)	96.4 (2.1)	91.1 (3.1)	44.1 (2.9)	105.9 (1.9)	104.6 (0.4)	104.3 (1.2)	107.0 (0.5)	101.7 (1.9)
	FA-TEA-45	99.0 (2.6)	97.2 (3.0)	95.2 (0.6)	93.6 (2.5)	98.9 (3.1)	92.7 (4.3)	95.5 (2.0)	98.3 (0.6)	98.9 (1.3)	98.3 (0.6)	92.2 (1.8)
	AA-TEA-58	–	–	–	–	–	96.5 (3.1)	105.2 (2.5)	–	–	101.5 (0.5)	99.5 (1.7)
	AA-TEA-84	–	–	–	–	–	97.4 (2.8)	100.3 (1.9)	–	–	103.7 (0.7)	96.8 (2.0)
	FA-DIPEA-45	–	–	–	–	–	101.2 (2.5)	101.1 (1.6)	–	–	99.9 (0.4)	100.9 (1.1)
DUL	FA-NH3-35	102.6 (3.2)	103.2 (3.5)	–	–	–	–	–	–	–	–	–
	FA-NH3-45	93.4 (3.3)	96.0 (2.2)	–	–	–	–	–	–	–	–	–
	FA-TEA-35	104.4 (5.1)	96.6 (2.3)	109.8 (1.2)	103.7 (6.6)	97.2 (2.0)	81.1 (2.6)	114.3 (1.7)	106.6 (0.4)	106.2 (1.2)	108.8 (0.6)	104.1 (1.5)
	FA-TEA-45	101.1 (4.9)	100.6 (2.4)	99.8 (0.9)	98.6 (5.4)	98.4 (1.8)	99.5 (1.6)	98.0 (1.6)	96.8 (0.9)	98.3 (1.1)	98.1 (0.8)	97.1 (2.2)
	AA-TEA-58	–	–	–	–	–	100.6 (1.4)	98.9 (1.5)	–	–	99.1 (0.7)	101.3 (1.7)
	AA-TEA-84	–	–	–	–	–	98.1 (1.5)	96.9 (1.8)	–	–	100.9 (0.6)	96.9 (2.3)
	FA-DIPEA-45	–	–	–	–	–	103.1 (1.8)	104.9 (1.9)	–	–	102.3 (0.7)	104. 1 (2.1)

Table 2 (*Continued*)

Analyte	Buffer	Cartridge type											
		Chromabond C18ec (1000 mg)	Chromabond C18ec (500 mg)	Supelclean LC-18 (500 mg)	Discovery DSC-18 (500 mg)	LiChrolut RP-18 (500 mg)	Bakerbond Octadecyl (200 mg)	Zorbax C18 (200 mg)	Bakerbond Phenyl (500 mg)	Strata-X RP (200 mg)	Oasis HLB (60 mg)	Bakerbond SDB-1 (200 mg)
ALI	FA-NH3-35	103.4 (0.9)	98.6 (0.9)	–	–	–	–	–	–	–	–	–
	FA-NH3-45	92.9 (1.2)	95.9 (1.3)	–	–	–	–	–	–	–	–	–
	FA-TEA-35	105.5 (0.7)	100.9 (1.2)	103.7 (0.7)	96.0 (4.9)	100.5 (0.8)	78.9 (1.3)	107.1 (1.4)	107.0 (0.7)	103.1 (1.0)	108.4 (0.4)	0.0
	FA-TEA-45	101.9 (0.9)	102.3 (1.1)	101.9 (0.6)	102.5 (4.2)	101.8 (0.7)	101.9 (0.9)	101.6 (1.4)	101.3 (0.5)	97.2 (1.4)	101.5 (0.3)	5.5 (4.3)
	AA-TEA-58	–	–	–	–	–	102.6 (1.2)	103.5 (1.5)	–	–	100.7 (0.5)	41.5 (2.0)
	AA-TEA-84	–	–	–	–	–	98.3 (1.3)	97.1 (1.2)	–	–	99.6 (0.2)	43.2 (1.8)
	FA-DIPEA-45	–	–	–	–	–	103.7 (1.4)	103.3 (1.6)	–	–	100.2 (0.3)	54.2 (1.9)
NHDC	FA-NH3-35	100.9 (3.1)	103.9 (2.9)	–	–	–	–	–	–	–	–	–
	FA-NH3-45	94.7 (3.4)	100.2 (3.5)	–	–	–	–	–	–	–	–	–
	FA-TEA-35	105.0 (4.9)	101.2 (3.6)	114.8 (1.3)	108.5 (5.4)	101.0 (3.3)	94.9 (1.2)	112.3 (2.0)	108.7 (0.5)	107.8 (1.3)	111.6 (0.9)	102.3 (3.2)
	FA-TEA-45	103.9 (4.1)	103.8 (3.9)	103.0 (1.4)	102.1 (2.1)	101.9 (2.6)	102.8 (1.0)	101.9 (1.8)	99.9 (1.1)	102.2 (1.1)	101.9 (0.8)	98.8 (1.8)
	AA-TEA-58	–	–	–	–	–	103.2 (1.1)	106.7 (2.3)	–	–	104.3 (1.4)	96.2 (1.9)
	AA-TEA-84	–	–	–	–	–	104.7 (1.3)	101.8 (1.4)	–	–	105.8 (1.3)	95.7 (2.30
	FA-DIPEA-45	–	–	–	–	–	106.8 (1.7)	106.2 (1.9)	–	–	100.9 (0.6)	101.6 (1.5)
NEO	FA-NH3-35	101.8 (3.1)	104.1 (3.2)	–	–	–	–	–	–	–	–	–
	FA-NH3-45	102.5 (2.7)	98.4 (3.0)	–	–	–	–	–	–	–	–	–
	FA-TEA-35	105.6 (2.6)	101.3 (2.9)	109.0 (1.2)	102.6 (0.5)	100.5 (1.7)	94.9 (1.0)	115.3 (1.7)	110.4 (0.5)	109.3 (0.8)	111.3 (1.0)	73.5 (3.7)
	FA-TEA-45	102.7 (2.4)	103.1 (2.5)	102.5 (1.1)	102.0 (0.6)	101.9 (1.3)	101.8 (1.2)	102.8 (1.9)	100.9 (1.1)	101.9 (1.0)	102.6 (0.9)	82.4 (4.9)
	AA-TEA-58	–	–	–	–	–	102.8 (1.2)	106.6	–	–	102.1 (0.8)	107.8 (5.3)
	AA-TEA-84	–	–	–	–	–	102.0 (1.3)	97.8	–	–	104.5 (1.2)	100.2 (1.8)
	FA-DIPEA-45	–	–	–	–	–	106.9 (2.1)	106.4	–	–	101.6 (0.6)	105.2 (2.1)

2.5. HPLC–MS analysis

The chromatographic separation of the sweeteners was achieved using a gradient elution, which was programmed with the initial mobile phase at 0% A, 100% B held for 4 min, ramped to 47% A, 53% B at 14 min. At 20 min, the mobile phase was ramped to 100% A, 0% B and held until 24 min, then isocratic for 2 min. Subsequently, the mobile phase returned to initial conditions in 2 min and then equilibrated for 10 min. Complete resolution of all analytes was obtained within the total run time of 36 min (Fig. 2). The mobile phase flow rate was 0.5 mL/min and the temperature of the column compartment was set to 22 °C. MS detection was performed in a time-scheduled SIM mode, using electrospray interface operating in the negative ion mode as depicted in Table 1. The operating parameters for ESI/MS in negative mode were as follows: capillary voltage 4000 V, nebulizer gas pressure 350 kPa, drying gas temperature 300 °C and drying gas flow rate 12 L/min.

The recoveries were calculated according to the following formula:

$$\%R = 100\% \left(\frac{A_{SPE}/IS_{SPE}}{A/IS} \right)$$

where A is the peak area of an analyte obtained after injecting four times diluted working standard; IS is the peak area of an IS obtained after injecting four times diluted working standard; A_{SPE} is the peak area of an analyte obtained after injecting SPE extract; IS_{SPE} is the peak area of an IS obtained after injecting SPE extract.

3. Results and discussion

3.1. The influence of pH and buffer type on the recovery of analytes

First experiments were conducted under the conditions given in the literature [12] but using FA-AM-45 buffer (please note that last two digits in buffer abbreviation denote its pH, *i.e.* 45 means pH 4.5, 35 means pH 3.5 and so on), instead of FA-TEA-45 (for the MS compatibility reason, as explained earlier). Under these conditions, Chromabond® C18ec (1000 mg) cartridges were able to retain all but one of the analytes, with the efficiency of around 95% and higher (Table 2). Low (85%) recovery of ACS-K was very difficult to explain since previously published data [12] indicates that the breakthrough volume of this type of cartridge for ACS-K should be around 30 mL. In our case significant amounts of ACS-K were detected in a liquid leaving the cartridge already after passing of 5 mL of a solution (including washing step). In order to solve this problem another set of SPE experiments was conducted. This time a buffer with lower pH value (FA-AM-35) was tested. Since ACS-K is a substance of acidic nature it was expected that lowering the pH of the sample will improve its recovery by promoting formation of electrically neutral, more hydrophobic form. Indeed, use of FA-AM-35 buffer resulted in 12% increase (from 85 to 97%) of ACS-K recovery. The breakthrough volume was higher at pH 3.5, but still far lower than reported by others [17]. One of the laboratories, mentioned in [17], obtained quantitative recovery of ACS-K using an SPE cartridge containing just 500 mg of the sorbent. In our case only 37% of ACS-K was recovered using Chromabond® C18ec (500 mg) cartridges. Clearly the pH of the sample is not the only factor influencing the retention of analytes.

Use of ammonia instead of triethylamine was the only difference between our experimental conditions and those described in [12]. Therefore, it was decided to verify whether the type of the buffer used during SPE could affect the results. A set of SPE experiments was conducted using FA-TEA-35 buffer and two types of Chromabond® C18ec cartridges (1000 and 500 mg).

Table 3
Characteristics of SPE sorbents used for extraction of high-intensity sweeteners.

Sorbent	Structure	Porosity (Å)	Particle diameter (μm)	End-capping	Carbon load (% C)	Surface area (m^2/g)
Chromabond C18ec	Silica–$(CH_2)_{17}$–CH_3	60	45	Yes	14	500
Bakerbond Octadecyl	Silica–$(CH_2)_{17}$–CH_3	60	40	Yes	17–18	n.a.
Bakerbond Phenyl	Silica–C_6H_5	60	40	Yes	10.6	n.a
Zorbax C18	Silica–$(CH_2)_{17}$–CH_3	80	50	No	11.1	n.a.
Discovery DSC C18	Silica–$(CH_2)_{17}$–CH_3	70	50	Yes	18	480
Supelclean LC-18	Silica–$(CH_2)_{17}$–CH_3	60	45	Yes	11.5	475
LiChrolut RP-18	Silica–$(CH_2)_{17}$–CH_3	40–63	–	–	–	–
Bakerbond SDB-1	PS–DVB–EVB	300	40	–	–	1060
Oasis HLB	PS–DVB–NVP	80	30	–	–	800
Strata-X	PS–DVB	85	33	–	–	800

PS: polystyrene; DVB: divinylbenzene; NVP: N-vinylpyrrolidone; EVB: ethylvinylbenzene.

TEA-based buffer solutions were found to be superior over ammonia ones. For both types of cartridges, complete recovery of all sweeteners was achieved. Interestingly, further investigation revealed that pH-recovery relationship for this type of buffer is reversed in comparison to ammonia based buffer. Apparently, TEA acts as an ion-pairing (or salt-forming) agent facilitating stronger interactions between the sorbent and sweeteners. At higher pH, higher fraction of weakly acidic sweeteners exists in an ionised state capable to form complexes with TEA. It seems that retention of such complexes occurs mainly due to hydrophobic interaction of three ethyl groups of TEA with the sorbent. This theory was confirmed by replacing TEA with another tertiary amine, N-substituted with longer aliphatic chains. Application of N,N-diisopropylethylamine based buffer (FA-DIPEA-45) seriously improved extraction efficiency of ACS-K and CYC when cartridges filled with just 200 mg of sorbent (Bakerbond Octadecyl, Zorbax C18) were used (Table 2).

3.2. Applicability of selected SPE sorbents for extraction of sweeteners

A comparative study on several commercially available C_{18}-bonded silica, phenyl-bonded silica and polymeric SPE sorbents (Table 3) was conducted in order to evaluate the feasibility of these sorbents for extraction of studied sweeteners from aqueous solutions. The complete set of results is shown in Table 2.

Nowadays, C_{18}-bonded silica sorbents are probably the most commonly employed sorbents for SPE. There are many commercially available SPE cartridges filled with these sorbents and sold under different brand names. Their specifications are similar and, according to our experience, all of them are suitable for extraction of high-intensity sweeteners from aqueous matrices.

Bakerbond Phenyl SPE cartridges can efficiently retain all of the studied sweeteners, when operated under optimal or close to optimal conditions. It is worth to note that when used under sub-optimal conditions (*i.e.* in combination with FA-TEA-35 buffer) this sorbent retains SAC much better than ACS-K and CYC. Probably, due to the presence of an aromatic ring in SAC's structure, interact with sorbent's phenyl groups by the formation of π–π bonds. Interestingly, similar behaviour can be observed in the case of Zorbax C18 which may suggests that phenyl groups are present within the bonded phase of this sorbent.

Another group of sorbents included in our study were polymeric sorbents. With some remarks, they were found to be suitable for extraction of sweeteners from aqueous solutions. All of the studied compounds were quantitatively retained using Strata-X polymeric RP sorbent. Oasis HLB cartridges gave lower recoveries for ACS-K and CYC (74 and 41%, respectively), although it must be noted that these cartridges were filled with only 60 mg of sorbent. This is over three times less than in case of other cartridges. An experiment with a cartridge filled with 300 mg of Oasis HLB sorbent (collected from five 60 mg cartridges) showed quantitative recovery of all sweeteners under the study. Also, use of DIPEA based buffer significantly improves extraction efficiency for ACS-K and CYC.

Very interesting results were obtained using SDB-1 cartridges and FA-TEA-35 buffer. The recoveries of most of the compounds (including the "difficult" ones, *i.e.* ACS-K, SAC and CYC) were very high, while ASP and ALI were completely lost, and for NEO recovery was only 74%. ASP, ALI and NEO are dipeptide sweeteners and for some, unclear for the moment, reasons they do not interact efficiently with an SDB-1 sorbent.

Since the retention of analytes on SDB-1 relies almost exclusively on hydrophobic analyte–sorbent interactions, relatively high polarity of dipeptide sweeteners could be responsible for the observed phenomenon. To verify this hypothesis further experiments have been conducted. Two additional buffers of higher pH (AA-TEA-58 and AA-TEA-84) were applied throughout the SPE procedure. As it can be seen in Fig. 3, extraction efficiency increases with the increase of pH. Complete recovery of NEO can be achieved at pH of 5.8. Nevertheless, even at the highest pH tested, ASP and ALI can be recovered with only 26 and 43% efficiency, respectively.

The improved retention of dipeptide sweeteners at higher pH's seems to be related to their ionisation degree in the solution. All these sweeteners have a pKa_1 (–COOH) value around 3. In case of buffer adjusted to pH 3.5 or 4.5, percent of ionised species in solution is smaller compared to buffers adjusted to pH 5.8 and 8.4. Since only ionised moieties can form non-polar, strongly retained, hydrophobic ion-pairs, better recoveries are observed at higher pH values. Relatively high recovery of NEO even at pH 3.5 is most probably caused by the presence of a non-polar neohexyl group and the lack of primary amino group in its structure.

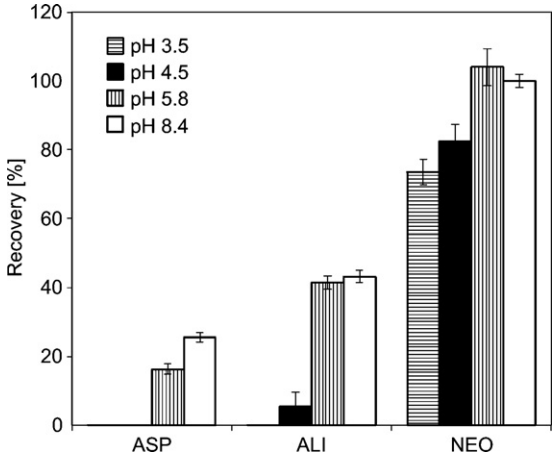

Fig. 3. Recovery of dipeptide sweeteners using SDB-1 SPE cartridges.

4. Conclusions

A thorough study on the suitability of different types of commercially available SPE cartridges for the extraction of high-intensity sweeteners from aqueous solution has been carried out. All of tested C_{18}-bonded silica sorbents are suitable for extraction of high-intensity sweeteners from aqueous matrices, providing that appropriate mass of sorbent (500 mg seems to be the right choice for most applications) is used. Performance of other types of cartridges (sorbents) varies. In general, they behave similarly to C_{18}-based ones, though, unusual selectivity towards dipeptide sweeteners has been noted in case of SDB-1 cartridges. This phenomenon can be employed for selective isolation of aspartame and alitame from the mixture of sweeteners. This can be important when a less efficient separation technique (compared to HPLC) is employed before final determination or when the presence of other sweeteners would interfere with the final detection.

The retention of analytes depends strongly on the composition of sample. Proper choice of the buffer (composition and pH) is essential for obtaining quantitative retention of sweeteners. The ion-pair reagent triethylamine, as a component of the buffer, greatly improves their retention by all types of sorbents studied. Even better results can be achieved by replacing triethylamine with diisopropylethylamine.

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