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Development of a quantitative high-performance thin-layer chromatographic method for sucralose in sewage effluent, surface water, and drinking water

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ABSTRACT

Sucralose, a persistent chlorinated substance used as sweetener, can already be found in waste water, and various countries focused on the release of sucralose into the aquatic environment. A quantitative high-performance thin-layer chromatography (HPTLC) method, which is orthogonal to existing methods, was developed to analyze sucralose in water. After sample preparation, separation of up to 17 samples was performed in parallel on a HPTLC plate silica gel 60 F_{254} with a mixture of *iso*propyl acetate, methanol and water (15:3:1, v/v/v) within 15 min. Due to the weak native UV absorption of sucralose (≤200 nm), various post-chromatographic derivatization reactions were compared to selectively detect sucralose in effluent and surface water matrices. Thereby *p*-aminobenzoic acid reagent was discovered as a new derivatization reagent for sucralose. Compared to the latter and to β-naphthol, derivatization with aniline diphenylamine *o*-phosphoric acid reagent was slightly preferred and densitometry was performed by absorbance measurement at 400 nm. The limit of quantification (LOQ) of sucralose in drinking and surface water was calculated to be 100 ng/L for a given recovery rate of 80% and the extraction of a 0.5 L water sample. The sucralose content determined in four water samples obtained during an interlaboratory trial in 2008 was in good agreement to the mean laboratory values of that trial. According to the *t*-test, which compares the results with the target value, the means obtained by HPTLC were not significantly different from the respective means of six laboratories, analyzed by HPLC–MS/MS or HPLC–TOF-MS with the use of mostly isotopically labeled standards. The good accuracy and high sample throughput capacity proved HPTLC as a well suited method regarding quantification of sucralose in various aqueous matrices.

1. Introduction

Sucralose (E 955) is a free flowing, non-hygroscopic, relatively heat resistant sweetener showing a sweetening intensity of 600–650 times as compared to sucrose without any after taste and calories. Since 1998 it has got approval by the regulatory agencies of over 80 countries [1,2] for use in thousands of food, consumer and pharmaceutical products. Sucralose is a trichlorosucrose (Fig. 1) produced via a selective chlorination of sucrose making it almost non-absorbable by the human intestines. Fortunately, despite being a chlorinated product, the toxic relevance of sucralose to humans is judged to be low [3]. Nevertheless, due to the lack of scientific evidence regarding harmful effects, a daily intake of not more than 15 mg/kg bodyweight is recommended by the European Union Scientific Committee. As it is not metabolized and thus excreted in the urine and feces the persistent chlorinated substance can meanwhile be found not only in waste water, but also in the natural water sources. It could get enriched in aquatic ecosystems, potentially contaminate drinking water though leakage or have an undesirable influence on sensitive organisms. In contrast to aqueous media, metabolism rates were observed in soil, but strongly depending on the bacterial assemblage [2,4].

In 2007, it was reported as being present in treated sewage effluents in μg/L concentrations and also in surface waters in Norway and Sweden. The sucralose findings were up to 11 μg/L in waste water treatment effluents and up to 3.5 μg/L in surface waters [4]. As waste water treatment effluents are the most likely conduit to natural water sources, further countries started to focus on the release of sucralose into the aquatic environment. In a screening of EU surface waters it was found in concentrations up to 1 μg/L in samples from UK, Belgium, the Netherlands, France, Switzerland, Spain, Italy, Norway and Sweden. In samples from Germany and Eastern Europe the concentration was minor [5]. In Switzerland sucralose findings in sewage effluents were up to 9 μg/L [6]. In USA, the highest findings in sewage effluents were reported to be 120 μg/L [2] as well as 1.8 μg/L in surface water and 2.4 μg/L in ground water [7]. Pragmatically, as sucralose is highly persistent in water, it is in discussion as a tracer for hydro-

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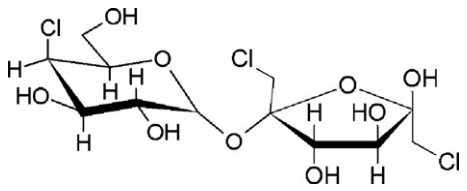

Fig. 1. Structure formula of sucralose (1,6-dichloro-1,6-dideoxy-β-D-fructofuranosyl-(2 → 1)-4-chloro-4-deoxy-α-D-galactopyranoside).

logic studies of wastewater and their impact on surface and ground water [6,7].

HPLC–MS(/MS) [5,8,9], HPLC-TOF [7] and GC–MS [2] were preferably used as analytical methods in the ultra-trace range after solid phase extraction (SPE). The focus was widened to other sweeteners, which were found in waste water. Like sucralose, especially acesulfame-K (E 950) can be found to a higher content in sewage effluent due to its persistence and its very low elimination in waste water treatment plants compared to other sweeteners [6,9]. The elimination rates of waste water treatment plants were reported to be about 40% for acesulfame-K and only 20% [9,10] or even below 10% for sucralose [4].

Although different analytical methods exist, the objective of this study was to find out whether HPTLC-Vis could be an orthogonal screening method for quantification of sucralose in environmental water samples. HPTLC-Vis was demonstrated for food samples with a high matrix load to be a reliable quantitative method, and at the same time, allow for high sample throughput and cost-effectiveness [11,12]. Such a simplified method would be appreciated for a wide screening to find key sources of the sucralose release into the aquatic nature. This is of great importance as sucralose's occurrence, distribution and impact is still unclear once it enters sensitive aquatic ecosystems, in which this anthropogenic compound should not be present.

2. Materials and methods

2.1. Reagents

Sucralose was provided by Tate & Lyle Specialty Sweeteners (Reading, England). For SPE, different (styrene-)divinylbenzene copolymer materials were used, like Bond Elut PPL (500 mg/3 mL, Varian, Darmstadt, Germany), Isolute 101 (200 mg/6 mL, Biotage, Düsseldorf, Germany) as well as the mixed-mode Evolute CX-50 (200 mg/3 mL, Biotage, Düsseldorf, Germany) and the hydrophilic (N-vinylpyrrolidone)-lipophilic (divinylbenzene) balanced Oasis HLB (200 mg/6 mL, Waters, Eschborn, Germany). For additional purification of the extracts, Bond Elut NH_2 (200 mg/3 mL, Varian) and the multi-mode Isolute M-M (500 mg/3 mL, Biotage) were employed. HPTLC plates silica gel 60 F_{254} (20 × 10 cm) were supplied by Merck (Darmstadt, Germany). Di-potassium hydrogen phosphate (Fluka, Buchs, Switzerland) used for plate impregnation was of analytical grade. For pH adjustment of the water samples, sodium hydrogen carbonate (purity 99.5%) was obtained from Merck. Ultra pure water was produced by Synergy System (Millipore, Schwalbach, Germany). Acetonitrile (chromatography grade) was obtained from vwr (Darmstadt, Germany). Other solvents (technical grade) were purchased from BASF (Ludwigshafen, Germany) and distilled prior to use. For derivatization, 2-naphthol, *p*-aminobenzoic acid, diphenylamine (all Merck), aniline (Fluka) and primuline (Waldeck, Münster, Germany) were used.

2.2. Plate pretreatment

Plate pre-treatment can be performed for a whole plate package in advance. HPTLC silica gel plates were washed by predevelopment with methanol, followed by drying at 100 °C for 15 min. For some experiments, the plates were additionally impregnated with di-potassium hydrogen phosphate solution (0.1 M, 3.4 g were dissolved in 200 mL of a mixture of methanol and water 3:2, v/v) with a dipping speed of 3 cm/s and a dipping time of 8 s using the TLC Immersion Device III (CAMAG, Muttenz, Switzerland), followed by drying on the TLC Plate Heater III (CAMAG) at 120 °C for 20 min and cooling-down in a desiccator.

2.3. Standard solution

As stock solution, 5.0 mg of sucralose were dissolved in 50.0 mL methanol (100 ng/μL). The stock solution was diluted 1:10 with methanol to obtain the standard solution (10 ng/μL), which was used for calibration and spiking of water samples with sucralose.

2.4. Origin of samples

Drinking water was taken from the domestic tap water supply at the Institute. The surface water was assayed at the river Körsch in Plieningen (N 48°42′15.69″, E 9°12′45.11″) at 0.2 m water depth and the sewage effluent at the waste water treatment plant in Plieningen (N 48°42′37.02″, E 9°13′59.02″) at 0.2 m water depth, all collected in carboys. If necessary, filtration of the samples was performed through a glass funnel lined with filter paper. Water samples were spiked with sucralose at a concentration of 1 μg/L. Therefore 50 μL of the standard solution (10 ng/μL) were added to a ca. 0.3 L portion of the sample, filled up to 0.5 L and shaken.

Effluent from a sewage wastewater treatment (sample A, Henriksdal in Stockholm, Sweden) and the same sample spiked with sucralose at a level of 37% of the original sucralose content (sample B, spike addition of 2.2 μg/L) and two identical surface water samples from the inner archipelago of Stockholm (at Nacka Strand, samples C and D) were obtained during an interlaboratory study in June 2008, already filtered (glass fibre filter GF/C, Whatman) and acidified with HCl to pH 3 [8]. The four ca. 1 L samples (A–D) were received in a still frozen state and stored at −20 °C until usage two years later.

2.5. Extraction of samples

The pH value of acidic water samples was adjusted to pH 8 with sodium hydrogen carbonate (20 mM). Different SPE adsorbents were investigated. The cartridges were positioned on a 12-port SPE vacuum manifold (Visiprep, Supelco, Bellefonte, USA) and conditioned with 3 mL methanol and 3 mL water. 0.5 L water sample flew through the still wet adsorbent using a water suction pump (flow rate 10 mL/min). For supply, a 60 mL reservoir was positioned by an adapter on top of the cartridges. The adsorbent was dried by suction of air for 30 min. Elution by gravity followed with 1 mL methanol after 5 min residence time and was repeated twice. For almost complete eluate recovery, after the last elution portion a slight suction was performed for 15 s using a water suction pump. Finally, the combined eluate (ca. 3 mL) was purified through the amino propyl or multi-mode phase. Again, for almost complete eluate recovery, the SPE material was additionally eluted with 2 mL methanol, which was collected with the purified extract. After concentration to 2 mL by gentle evaporation under nitrogen (vapotherm basis mobil I, Barkey,

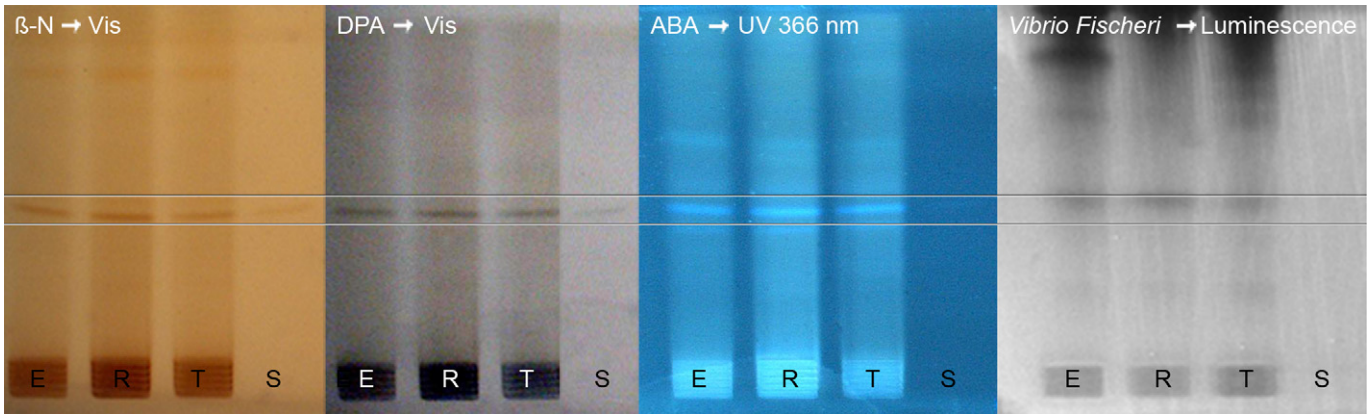

Fig. 2. Selective derivatization of sucralose (hR_F window marked) by immersion in β-naphthol sulfuric acid reagent (β-N, brown color of sucralose), aniline diphenylamine *o*-phosphoric acid reagent (DPA, grey color of sucralose), both documented at white light illumination, and *p*-aminobenzoic acid reagent (ABA, blue fluorescent band of sucralose) documented under UV 366 nm; immersion in *Vibrio fischeri* suspension did not show any response for sucralose (documented in the dark with the BioLuminizer); exemplarily spiked effluent (E), river (R) and tap water samples (T) as well as a standard track (S, 30 ng/band) were shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Leopoldshoehe, Germany), the extracts were transferred to 1.8 mL sampler vials.

2.6. Application

The solutions were sprayed as area (8 × 5 mm) with the Automatic TLC Sampler 4 (ATS4, CAMAG) allowing 17 tracks to be applied on one HPTLC plate of 20 × 10 cm (distance between bands 10 mm, distance from lower edge 8 mm, distance from the left side 20 mm). For five-point-calibration, volumes of 1, 5, 10, 15 and 20 μL of the standard solution were applied on the plate (10–200 ng/band for sucralose) over a calibration range of 1:20. A four-point calibration over a wider range (1:30, application of 1, 10, 20 and 30 μL, 10–300 ng/band for sucralose) was also suited. Typical volumes of sewage effluent extracts sprayed-on were 100 μL. For analysis of surface water and for determination of the limit of detection, 300 μL-volumes have been used. Area application and heating the ATS4 spray nozzle to 60 °C was used for application of the large volumes to accelerate spraying. This allowed the upscale of the dosing speed (150 nL/s) to 1000 nL/s. By doing so, a reduction by 70% of the regular application time was reached. Hence, the 100 and 300 μL volumes were applied in 1.7 and 5 min, respectively.

2.7. Chromatography

Development was performed in the Automated Developing Chamber 2 (CAMAG) at a relative air humidity of about 30% with a mixture of *iso*propyl acetate, methanol and water (15:3:1, v/v/v). The migration distance was 60 mm from the lower plate edge and the migration time was 15 min.

In case of start zone focusing, the sucralose located over the whole application area was front-eluted with acetonitrile–water 5:1 (v/v) to a sharp band up to a migration distance of 11 mm, which took just some seconds. Of course, drying in a stream of warm air followed for 1 min.

For the plate cleaning experiment, the plate was developed with either methanol or methanol–water 4:1 (v/v) up to a migration distance of 60 mm and dried in a stream of warm air.

2.8. Effect-directed detection by a bioassay (bioluminescence)

For bioluminescence detection the developed plate was automatically dipped at a speed of 3.5 cm/s and an immersion time of 0 s with the TLC Chromatogram Immersion Device III into the luminescent bacteria (*Vibrio fischeri*) suspension prepared according

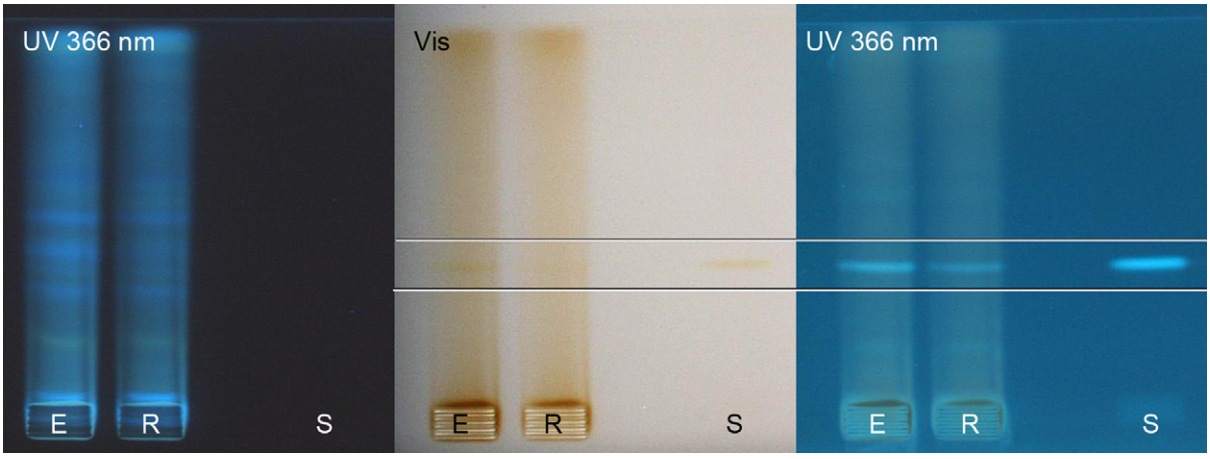

Fig. 3. *p*-Aminobenzoic acid reagent as new selective derivatization reagent for sucralose: the hR_F window of sucralose after the selective derivatization (two images right) is marked (yellow band at white light illumination, blue fluorescent band under UV 366 nm, depicted here for effluent (E, overspray 50 ng/zone) and river water samples (R, overspray 20 ng/zone) as well as standard (S, 300 ng/zone); for comparison, right after chromatography the image under UV 366 nm is shown (left), whereas the matrix was not visible under white light illumination (not depicted). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

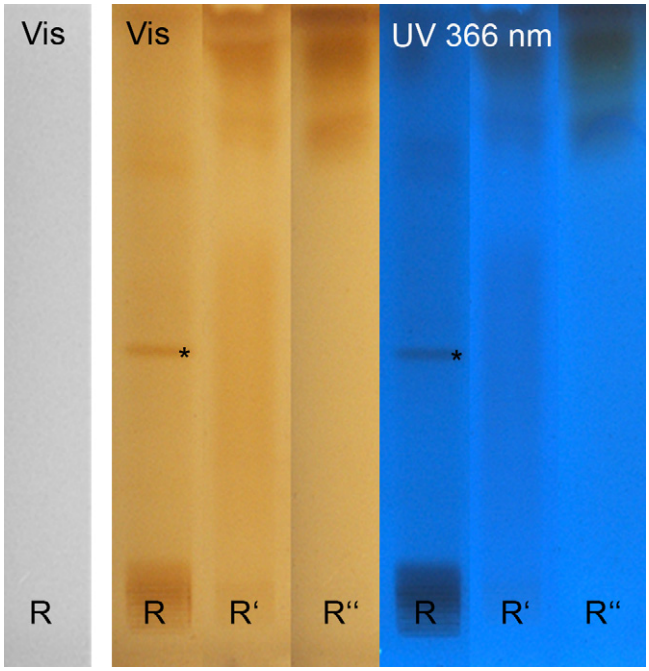

Fig. 4. Matrix visualization in a plate cleaning experiment: Matrix of river water (R, spiked with sucralose (marked*) was not visible at white light illumination on the chromatogram, but after derivatization (e.g., with β-naphthol sulfuric acid reagent documented at white light illumination and under UV 366 nm): the matrix of a developed plate partially remained on the adsorbent after cleaning with 100% methanol (R′); the cleaning was improved by adding water to the solvent (methanol–water 4:1, v/v) (R″).

to the Bioluminex assay protocol (ChromaDex, Boulder, CO, USA). For imaging, the HPTLC plate was placed into the compartment of the BioLuminizer (CAMAG) specially designed for bioluminescence detection. In the compartment the HPTLC plate was covered by a glass plate keeping the bacteria moist for a prolonged time. The images were captured with a cooled 16 bit high resolution CCD camera with an exposure time of 30 s. Over a period of 24 min, 8 images were recorded to monitor time-dependent changes.

2.9. Post-chromatographic derivatization

For the 2-naphthol sulfuric acid reagent, 2 g 2-naphthol were dissolved in 180 mL ethanol and 12 mL sulfuric acid (50%) were added dropwise to avoid an accelerated exothermal reaction. The reagent stored in the refrigerator was stable for at least a year.

For the aniline diphenylamine *o*-phosphoric acid reagent, 1.5 g aniline and 1.5 g diphenylamine were dissolved in 150 mL methanol and 15 mL *o*-phosphoric acid was added dropwise. The reagent stored in the refrigerator was stable for at least one month. After several weeks of usage, the specific blue fluorescence of sucralose might be impeded by increased reagent concentration due to solvent evaporation.

For the *p*-aminobenzoic acid reagent, 1 g 4-aminobenzoic acid was dissolved in 36 mL pure acetic acid, then, 40 mL water, 2 mL phosphoric acid (85%) and 120 mL acetone were added. This reagent stored in the refrigerator was stable for at least one month.

Post-chromatographic derivatization was performed with the TLC Immersion Device III (CAMAG) using a vertical speed of 2.5 cm/s and no immersion time (0 s), followed by heating the plate on the TLC Plate Heater III at 120 °C. Sucralose was visible after 3 min as brownish zone using the 2-naphthol sulfuric acid reagent, after 5 min as yellowish zone using the *p*-aminobenzoic acid reagent and after 20 min as gray-bluish zone using the aniline diphenylamine *o*-phosphoric acid reagent. With the latter two reagents sucralose was not only visibly but also showed a blue fluorescence if inspected under UV 366/>400 nm.

2.10. Documentation

Plate images were documented by DigiStore2 Documentation System (CAMAG) consisting of the illuminator Reprostar 3 and the Baumer optronic DXA252 digital camera. The exposure time for the plate derivatized with the 2-naphthol sulfuric acid, *p*-aminobenzoic acid and aniline diphenylamine *o*-phosphoric acid reagent was 7 ms (white light, reflectance and transmission mode), 700 ms and 1300 ms (both UV 366, reflectance mode), respectively. All images were captured at a gain of 1. Data obtained was processed with winCATS software, version 1.4.5.2027 (CAMAG).

2.11. Quantification by densitometry

Densitometric evaluation was performed with the TLC Scanner 3 (CAMAG) via peak height or area. The absorbance of sucralose was measured at 505 nm for the plate derivatized with the 2-naphthol sulfuric acid reagent and at 400 nm for the plate derivatized with the aniline diphenylamine *o*-phosphoric acid reagent. Fluorescence measurement of the latter plate and the plate derivatized with the *p*-aminobenzoic acid reagent was recorded at UV 366/>400 nm.

2.12. Identification by mass spectrometry

The sucralose zones on the non-derivatized HPTLC plate were marked. The *x*-axis coordinate was available through the application pattern and the *y*-axis was assured by comparison with a cut-off and derivatized sucralose standard track on the outer side. For recording of electrospray ionization (ESI) mass spectra by a single-quadrupole mass spectrometer (MSD, Agilent, Waldbronn, Germany), respective zones were directly eluted with the TLC–MS Interface (CAMAG) into the ESI spray chamber using methanol–ammonium formiate buffer (10 mM, pH 4) 19:1 (v/v) at a flow rate of 0.1 mL/min (HP 1100 pump, Agilent). The capillary voltage for positive and negative ionization was set to be +4 kV and −4 kV, respectively. The nebulizer gas pressure was 20 psig, the drying gas temperature 300 °C, and drying gas flow rate 10 L/min. The mass spectra were recorded in the negative and positive ion full scan mode between *m*/*z* 300 and 500 using as fragmentator voltage 100 V, gain 1, threshold 10, and step size 0.25. For the recording of spectra from the derivatized plate, the full scan mode was recorded between *m*/*z* 160 and 500, and *m*/*z* 500 and 1000. Data processing for MS measurements was carried out with LC/MSD ChemStation software Rev. B. 02.01-SR2 (Agilent).

3. Results and discussion

3.1. Chromatographic method

First separation trials were based on the method used for food matrices [11], and sucralose was separated on the HPTLC plate silica gel 60 impregnated with di-potassium hydrogen phosphate using a mixture of acetonitrile and water 17:3 (v/v). On the not-impregnated HPTLC plate silica gel 60, a mixture of acetonitrile and water 17:0.5 (v/v) was used. As the water extracts were applied as area, a short focusing step was assumed to be necessary before chromatography. Besides acetonitrile, which is listed in the solvent selectivity group VI according to Snyder [13], and further representatives of the same group (e.g., *iso*propyl acetate), also other significantly different mobile phase selectivities were investigated to obtain the utmost possible resolution between water matrix and analyte, e.g., solvent mixtures based on tetrahydrofurane (selectivity group III) or dichloromethane (selectivity group V). Finally, a

Table 1
Overview and comparison of the performance data for analysis of sucralose in environmental water samples.

Methods for analysis of sucralose	Online SPE-HPLC–MS/MS	Automated SPE HPLC–MS/MS	SPE HPLC–TOF-MS	SPE HPLC–MS/MS	SPE GC–MS	SPE LC–TOF-MS	SPE HPTLC-Vis
Authors	Buerge et al. [6]	Loos et al. [5]	Brorström-Lundén et al. [4]	Scheurer et al. [9,14]	Mead et al. [2]	Ferrer et al. [7]	Morlock et al.
Sample matrix investigated	Surface/waste water	Drinking/surface/waste water	Surface/sewage water	Surface/waste water	Surface/coastal/waste water	Surface/sewage/ground water	Drinking/surface/sewage water
Spike level (μg/L)	20–100	1	1	1	0.1	50–10000	1
Use of internal standard	Sucralose-d_6	Sucralose-d_6	–	Sucralose-d_6	–	–	–
Recovery (%)	Not given	62 for drinking water 55 for surface water 26 for waste water	94 for sewage water	88 for drinking water 82 for surface water 48 for waste water	81 for ultrapure water 89 for surface water 26 for samples with high dissolved organic matter	73 (water source not specified)	84 for drinking water
Sample volume (mL)	500	400 (pH 7)	1000/200 (pH 3)	50 (pH 3)	200–34000	200	500
SPE adsorbent (g)	2 (stacked) Biobeads SM-2 (10 mL)	Oasis HLB (0.2)	Oasis HLB (0.2)	Bakerbond SBD 1 (0.2)	Oasis HLB (0.2 or 6)	Oasis HLB (0.5)	Bond Elut PPL (0.5)
Eluent volume (mL) and solvent (v/v)	5	6	7	3 × 3	Volume not given	5	3 × 1
	methanol plus 25 (10 + 10 + 5) dichloromethane	methanol	acetone-methanol 5:1	methanol	methanol	methanol	methanol
Clean up	Dichloromethane phase concentrated to dryness	Concentration step	Via Isolute-MM (0.3) eluted with acetone–methanol 5:1	Concentration to dryness	Drying with sodium sulphate	Concentration step	Purification through Bond Elut NH_2
	→5 mL water; thereof 0.5 mL for online SPE (Gemini C18) with opposite transfer onto column		→concentration to 1 mL	→solvent change: reconstitution in 0.5 mL HPLC solvents	→concentration to dryness		→concentration
			→Oasis MAX (0.15)		→100 μL derivatization reagent, 30 min/70°		
			→concentration		→concentration to dryness →taken up in dichloromethane		
Final extract volume (mL)	5	0.5	1	0.5	Volume not given	0.5	2
Runtime per sample (min)	25	30 or 40	30	30 for waste water (or 9)	ca. 50	30 + 10	5
Detection	MS/MS: ESI	MS/MS: ESI	TOF-MS: ESI	MS: ESI	IT-MS: EI	TOF-MS: ESI	Vis
LOD (ng/L)	10	10	–	10	<15	50	100

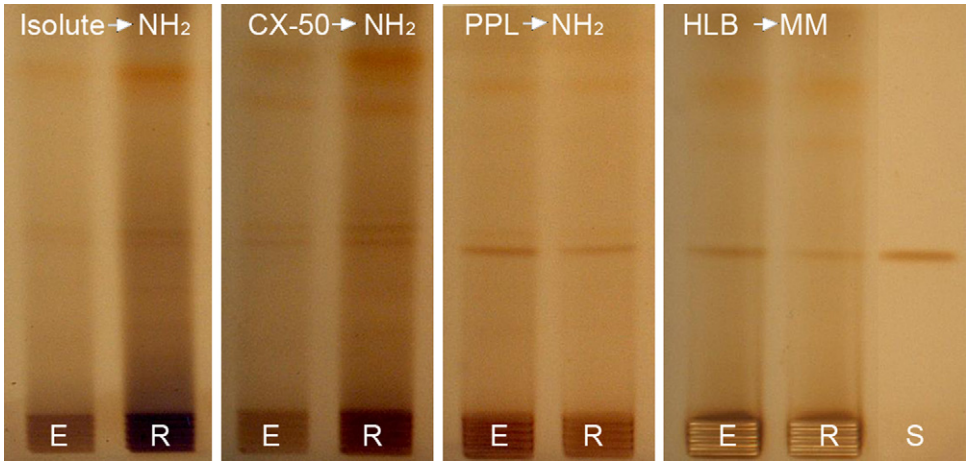

Fig. 5. Comparison of different SPE materials for sample preparation; spiked effluent (E) and river water (R) samples as well as a standard track (S, 300 ng/band) were exemplarily shown.

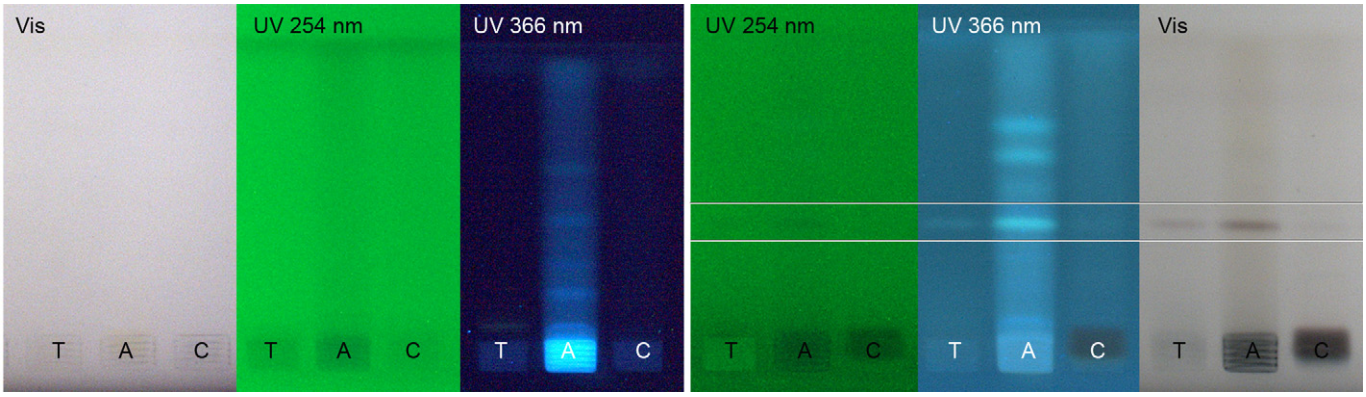

Fig. 6. Multiple information on a single plate shown for the effluent (A) and surface water (C) samples obtained from the interlaboratory trial in 2008 and a spiked tap water sample (T, 1 μg/L) used for recovery rate calculation, the hR_F window of sucralose after selective derivatization (three images right) is marked.

mixture of *iso*propyl acetate, methanol and water (15:3:1, v/v/v), separated best sucralose (hR_F 40) and detected it most selectively from matrix constituents (Fig. 2). Neither the plate impregnation nor the focusing step had been necessary to obtain this good separation as sucralose migrated in a β-front and was focused this way.

Performing selective derivatizations on the plate is a strong feature of planar chromatography because all chromatographic runs

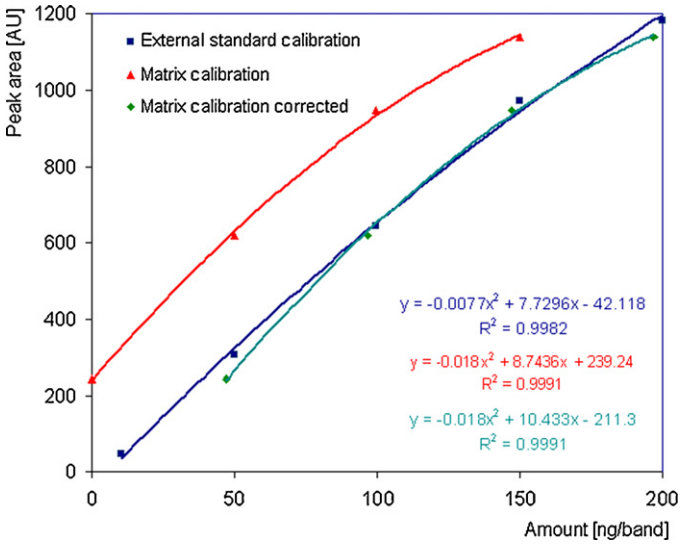

Fig. 7. Comparison of the calibration curves obtained by external standard calibration with that obtained by calibration in matrix and the matrix calibration corrected by the original finding; a similar slope confirms the use of an external standard calibration.

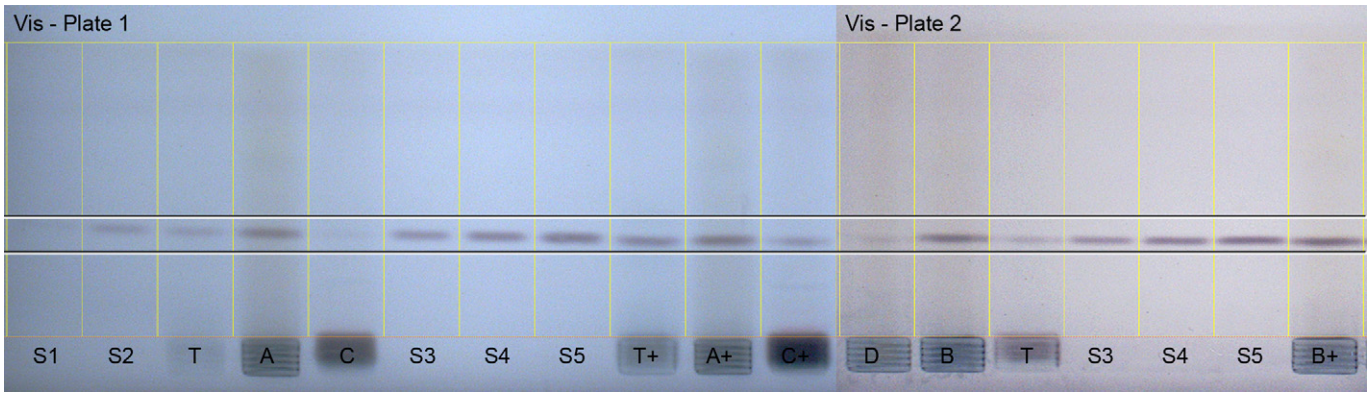

Fig. 8. Parts of plate images obtained for effluent (A and B) and surface water (C and D) samples obtained from the interlaboratory trial in 2008 and a spiked tap water sample (T, 1 μg/L) used for recovery rate calculation.

are derivatized simultaneously and selective derivatization dramatically increases the separation efficacy not seeing everything, but just the analyte and related compounds. Moreover, it enables the usage of more cost-effective detectors (UV/Vis/fluorescence). Hence, various post-chromatographic derivatization reagents according to [11] were tested for its selective detection of sucralose in drinking, surface and sewage effluent water. The preparation procedure for the 2-naphthol sulfuric acid reagent was simplified from a 4-step to 2-step based preparation (see Section 2.9) being much more convenient in contrast to [11]. 2-Naphthol sulfuric acid reagent was found to be superior due to the shorter heating time compared to the aniline diphenylamine *o*-phosphoric acid reagent, but the latter was found to be slightly superior with regard to selectivity (Fig. 2). Vis-spectra recorded confirmed the optimal measurement wavelength of 505 nm for the 2-naphthol sulfuric acid reagent and 400 nm for the aniline diphenylamine *o*-phosphoric acid reagent [11]. The *p*-aminobenzoic acid reagent was discovered to be a new selective derivatization reagent for sucralose leading to blue fluorescent zones under irradiation at UV 366 nm (Fig. 3), similar to the aniline diphenylamine *o*-phosphoric acid reagent. The detection by *V. fischeri* bacteria was not successful as sucralose did not show any response at a reasonably low concentration.

Fortunately in HPTLC, the high matrix load of the adsorbent is visible to the analyst. On a plate, the same samples were applied three times in three segments. After chromatography, the plate was cut in three identical parts. Plate part one was directly derivatized and documented. Plate part two was developed (cleaned) with methanol and plate part three with methanol–water 4:1, v/v. After cleaning, derivatization followed to visualize the matrix. It can clearly be seen that matrix partially remained on the adsorbent using methanol (Fig. 4, R′). The cleaning was improved by adding water to the solvent (Fig. 4, R″). For this study, waste water effluent and river water samples were applied as matrices, and both led to comparable images and to the same conclusion that the cleaning solvent should contain partially water to sufficiently purify the adsorbent. Potential matrix remained on the adsorbent cannot be made visible and proven in column chromatography. Only over the runs, it could be noticed as baseline or retention time shift or by any signal suppression. Hence in HPTLC, both, either the visible proof of the plate's re-use or the single-use of the stationary phase and the ability to have always fresh adsorbent are judged as being advantageous.

3.2. Sample preparation, recoveries and limit of detection

Local drinking and river water as well as sewage effluents, spiked with 1 μg/L sucralose each, were investigated as samples for optimization of the sample preparation procedure. Different (styrene-)divinylbenzene copolymer adsorbents were investigated for selective extraction of sucralose from matrix constituents, like the Bond Elut PPL, Isolute 101 as well as the mixed mode Evolute CX-50 and the Oasis HLB. Although HPTLC is capable for a high matrix load, a single SPE extraction, for example only Oasis HLB or only Bond Elut PPL, was not sufficient to selectively distinguish from matrix constituents despite mobile phase optimization trials. Thus, the eluate was additionally purified through either Bond Elut NH_2 or multi-mode Isolute M-M. For HPTLC purpose, the SPE extraction of sucralose from water samples was visually judged to be most selective using the Bond Elut PPL adsorbent followed by purification of the eluate through Bond Elut NH_2 (Fig. 5). The completeness of the elution was proven by collecting the eluate in separate fractions of 1 mL and applying those onto the plate.

The mean recovery of sucralose in drinking water and its standard deviation ($n = 3$) were determined to be $84 \pm 7\%$ at a 1 μg/L spike level. This recovery was comparable to Loos et al. [5] and Scheurer et al. [9], which reported $62 \pm 9\%$ ($n = 6$) and $88 \pm 0\%$ ($n = 3$) at the same spike level, respectively (Table 1). In river water and sewage effluents the mean recoveries can be lower due to increased matrix interferences. For example, recoveries of sucralose reported for surface water were about 55% [5] and $82 \pm 2\%$ ($n = 3$) [9] and for waste water about 26% [5] and $48 \pm 6\%$ [9]; all values were obtained at the same spike level. Any matrix influence can clearly be seen by the multi-detection capability of HPTLC (Fig. 6). The ease of multi-detection and further planar chromatographic hyphenations were recently described in a review [15].

Table 2
Comparison of the sucralose findings.

Sucralose in sample (ng/L)	Effluent water (A)	Effluent water (B)	Surface water (C)	Surface water (D)
Mean value by HPTLC-Vis	5863	7034	247	218
%RSD ($n = 2$)	4.5	1.0	27.8	13.0
Mean value of interlaboratory trial (outlier corrected)	5869	7302	186	200
%RSD ($n = 6$)	17	24	21	23
Bias (%)	0.1	3.7	−32.5	−9.0
Calculated *t*-value	0.04	5.42	1.25	0.90

For the constellation, i.e. 0.5 L sample being extracted at a supposed recovery rate of 80% and thereof 300 μL being applied on the plate, the limit of detection (LOD) was 100 ng/L (6 ng/band). If a 1 L sample is extracted the LOD will improve. The LOD is by a factor of 2 to 10 worse compared to other methods, which showed LODs of 10 ng/L [2,5,6,9] and 50 ng/L [7]. Of course, for HPLC–MS, the MS/MS and MS-TOF analyzers used show a high capability of detection. Nevertheless these detectors were highly expensive in asset costs and routine use.

3.3. Quantification

After derivatization with the 2-naphthol sulfuric acid or aniline diphenylamine *o*-phosphoric acid reagent and absorbance measurement at 505 or 400 nm, quantification was performed by peak height or area evaluation. For example, a 4-point calibration in the range from 10 to 300 ng/band of sucralose was suited to analyze a wide range of sucralose findings in water samples (100 ng/L to 5 μg/L). Starting with the limit of quantification, the calibration curve showed good performance characteristics (coefficient of correlation $r = 0.99991$, relative standard deviation %RSD = 1.3%). For comparison, the calibration in matrix was performed by overspraying the start zones of water extracts (multi-fold applied) with different volumes of sucralose standard solution. The slopes of both calibrations were similar (Fig. 7) and it can be concluded that the simple calibration by an external standard is suited for routine use. However, for low sucralose findings (<200 ng/L), the matrix calibration was preferred as it corrected any potential matrix influence.

3.4. Sample analysis

For proof of principle, the water samples obtained for an interlaboratory study in June 2008 were investigated. Due to manpower and time constraints, the samples were investigated not until May 2010. Based on the high stability of sucralose and its resistance against metabolism and transformation, samples were deemed to be sufficiently stable for the given purpose. The pH value of acidified Swedish water samples (1 L) was adjusted to pH 8 with 20 mM sodium hydrogen carbonate because matrix components, e.g., humic acids, might adsorb more strongly on the polymeric SPE adsorbent at acidic pH. Similar conclusions were drawn by Loos et al. [5], who noticed a signal suppression increase of 30% for waste water extracted at pH 3.

Findings by HPTLC (Fig. 8) were compared to the respective means of the interlaboratory study using HPLC–MS (Table 2). In the interlaboratory study [8], all laboratories used SPE for sample preparation. One laboratory, using HPLC-UV after pre-column derivatization with DNBC benzoylation, found highly reduced values (just about 10% of the target value) and was assigned as outlier. The residual 6 laboratories used HPLC–MS/MS or HPLC–TOF-MS. 5 out of 6 laboratories corrected the results by isotopically labeled standards.

The HPTLC results corrected by the recovery rate showed good reliability. The bias to the sucralose value specified by the interlaboratory study [8] was determined to be between 0.1 and 32.5%. According to the *t*-test, which compares the mean values of the interlaboratory study obtained by HPLC–MS/MS or HPLC–TOF-MS analyses with the mean values of the HPTLC findings, both values were statistically not significantly different. In all cases the calculated values were smaller than the *t*-table value of 9.93 ($P = 99\%$, two-sided, $\nu = 2$).

3.5. Identification by mass spectrometry

For recording of mass spectra, an elution head-based technique was used [16]. Regular settings were used and an optimization of the single parameters was not performed. First, from sucralose standard zones, mass spectra were recorded in both ionization modes and the typical 3-fold chlorine isotope pattern was clearly visible (Table 3). Thereby the formiate adduct was the most pronounced signal group in the negative ionization mode. The capability of detection (S/N of 3) was about 100 ng/zone using the standard settings. Then, respective zones of water samples supposed to be sucralose were recorded (Fig. 9).

Table 3
HPTLC–ESI-MS signal groups obtained from a sucralose standard zone recorded in the positive and negative ionization mode (bold: most intensive signal group).

Sucralose recorded in	Mass signal groups obtained (*m*/*z*)	Assignment
Positive ESI	419 421 423	$[M+Na]^+$
Negative ESI	395 397 399 **441** **443** **445** 493 495 497	$[M-H]^-$ **$[M+HCOO]^-$** $[M+H_2PO_4]^-$
After derivatization of sucralose with DPA		
Positive ESI	351 353 429 442 504	Unknown derivatization product

MS signals were also obtained from colored zones after derivatization with the aniline diphenylamine *o*-phosphoric acid reagent. Before, it was proven whether the derivatized zone is capable for elution. Therefore, an already derivatized and evaluated plate was cut 1 cm below the colored sucralose zone and used for chromatography with the elution solvent. The colored zone migrated with the solvent front, which was the proof that the online elution would transfer the colored analyte into the MS. The MS spectrum obtained showed an intense signal of the aniline diphenylamine *o*-phosphoric acid reagent at *m*/*z* 170 in the positive ESI mode, but after substraction of the plate background signal from the analyte zone, the signal group at *m*/*z* 351 and 353 were evident for repeated analyses ($n = 6$).

3.6. Comparison to methods in literature

Compared to literature the following steps were eased: An additional clean up through, e.g., OASIS MAX, for purification from humic acids was not relevant [4] and the concentration effort was mitigated: The eluate of about 4 mL was concentrated to 2 mL, which is only a concentration factor of 2 compared to a concentration factor of 5 [6], 7 [4], 10 [7], 12 [5] and 18 [9]. Any solvent change reported for HPLC–ESI-MS/MS was not necessary [9] because the solvent was evaporated after the planar chromatographic separation and does not interfere with derivatization or mass spectrometry.

Taking into account the most sophisticated online SPE-HPLC–MS/MS method [6], the total time per sample was reported to be within 2 h, wherein the chromatographic runtime was 25 min. As the HPTLC method is intended to find key sources of sucralose input, a high sample throughput is obvious. Up to 17 samples can be analyzed within 1.4 h (60 min for application, 15 min for separation, 5 min for derivatization and 5 min for evaluation). Hence, the HPTLC method takes only 5 min per sample. Having in mind that trouble at a small link in a fully online SPE-HPLC–MS/MS system will force

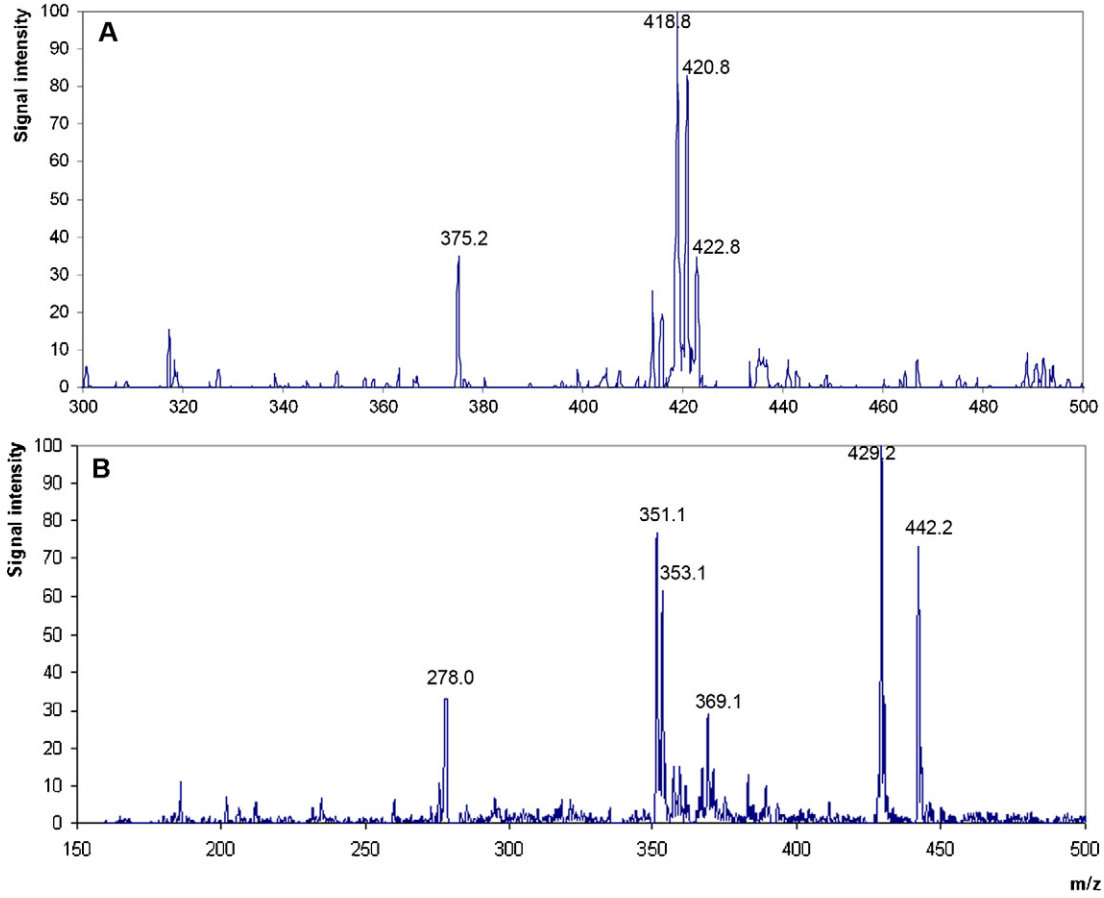

Fig. 9. HPTLC–ESI-MS spectra (positive mode) of sucralose found in water samples after chromatography (A) and after derivatization with the aniline diphenylamine *o*-phosphoric acid reagent (B), both after background substraction; the background spectrum was obtained by elution of a background blank at approximately the same migration distance; assignments see Table 3.

the whole system to stop, the step-automated HPTLC procedure and its instrumental flexibility is considered to be advantageous in routine use.

4. Conclusions

The good accuracy in matrix and the rapid chromatography, which is performed simultaneously for up to 17 samples, proves HPTLC as a well suited method regarding quantification of sucralose in various aqueous environmental matrices. For high sample throughput analysis, the HPTLC method (sample preparation not considered) takes less than 5 min per sample. The sucralose content determined in four specified reference water samples was in good agreement to the values obtained by HPLC–MS [8].

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