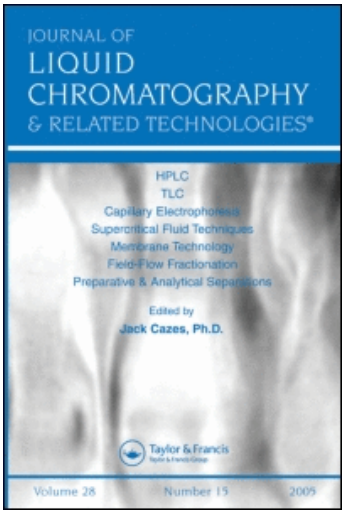

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A Simple and Reliable HPTLC Method for the Quantification of the Intense Sweetener Sucralose®

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ABSTRACT

This paper describes a simple and fast thin layer chromatography (TLC) method for the monitoring of the relatively new intense sweetener Sucralose® in various food matrices. The method requires little or no sample preparation to isolate or concentrate the analyte. The Sucralose® extract is separated on amino-TLC-plates, and the analyte is derivatized "reagent-free" by heating the developed plate for 20 min at 190°C. Spots can be measured either in the absorption or fluorescence mode. The method allows the determination of Sucralose® at the levels of interest regarding foreseen European legislation (>50 mg/kg) with excellent repeatability (RSD = 3.4%) and recovery data (95%).

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Key Words: Sucralose; Intense sweetener; Diode-array TLC; HPTLC; Fluorescence detection; Fluorescence enhancement; Reagent-free derivatization.

INTRODUCTION

Thin layer chromatography (TLC) and, especially, high performance thin layer chromatography (HPTLC) are flexible, fast, and inexpensive off-line separation techniques that are suitable for screening purposes in food analysis.[1] High performance thin layer chromatography allows a better separation due to a smaller particle size of the stationary phase. Sample application for separation is done directly on the stationary phase and, subsequently, a solvent flows through the stationary phase achieved by capillary force forming the mobile and the stationary phase. The separation step (A) and the densitometric detection (B) after evaporation of the mobile phase are two isolated steps. Densitometric scanning for quantitative purposes can be preferably performed by the use of a diode-array scanner/detector (DAD) by which simultaneous information about fluorescence and absorption spectra can be obtained directly on the HPTLC-plate. The fluorescence information is of great importance because it improves the selectivity of detection[2] and can offer extremely low detection limits.[3]

Sucralose® (4-chloro-4-deoxy-α-*D*-galactopyranosyl-1,6-dichloro-1,6-dideoxy-β-*D*-fructofuranoside) is an intense sweetener (Fig. 1) that shows a sweetening strength of 650 times more intensive than sucrose. Because of its strong sweet taste and high stability, Sucralose® has been introduced into the food market.[4] It is already approved in several countries for food additive use and is under discussion for approval in the European Union (EU). The limits in the EU are foreseen to range from 50 to 1000 mg/kg (or mg/L), depending on

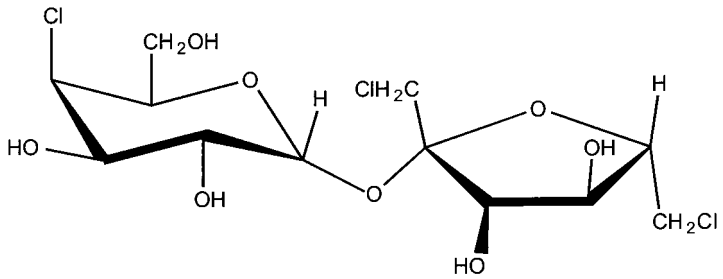

Figure 1. Structure of the intense sweetener 1,6-dichloro-1,6-dideoxy-β-*D*-fructofuranosyl-4-chloro-4-deoxy-α-*D*-galactopyranose (Sucralose®).

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the food items regulated. Sucralose® is a non-volatile substance similar to other carbohydrates, with a lack of any chromophore to facilitate the analysis.

Sucralose® is mostly determined in beverages and other food products by high performance liquid chromatography (HPLC), although the procedure is quite laborious and background interference may be encountered.[5] Some authors recommend refractive index detection to reduce interferences with other food components,[6–8] while several papers also describe pulsed amperometric detection of Sucralose® after anion-exchange chromatography (IC).[6,7] Both detection systems have their merits and limits, which are also discussed in the literature.[5–8]

The analysis of complex matrices, such as yogurt samples, jam, ready-to-eat desserts, and dry mix products (e.g., gelatin desserts or starch-based puddings), has been performed with a method that requires an extensive pre-cleaning procedure.[9] Another approach by capillary electrophoresis (CE) for the rapid determination of Sucralose® in beverages has been described recently.[10] Sucralose® in urine was analyzed by gas chromatography with flame ionization detection (GC/FID) after derivatization with oxime reagent in pyridine with a detection limit of 20 mg/L.[11]

Planar chromatography requires, in general, less sample clean-up because the TLC plates are relatively cheap and disposable. For this reason, we investigated the application of HPTLC for the determination of Sucralose® as an alternative method. The results are described in this paper.

EXPERIMENTAL

Reagents

Amino-bonded silica gel HPTLC-plates (10 × 10 cm) with fluorescent indicator (NH_2 F_{254S}) were obtained from Merck (Darmstadt, Germany). Acetonitrile and methanol were purchased from Fluka (Deisenhofen, Germany) and cetyltrimethylammonium bromide from Aldrich (Deisenhofen, Germany).

Sample Pretreatment

Beverages were applied directly on the HPTLC-plates, after standing for 10 min in an ultrasonic bath to remove carbon dioxide (if the beverages were carbonated). Samples of yogurt, jam, and powders from ready-to-eat desserts or starch-based puddings were mixed (5.0–20.0 g of sample material) with 20 mL of methanol–water (1 : 1). The mixture was shaken, treated for 10 min

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in an ultrasonic bath to extract the Sucralose®, and then filled with the solvent mixture to 50 mL. The obtained slurry was filtered and the filtrate applied directly on the HPTLC plate.

High Performance Thin Layer Chromatography Separation, Detection, and Densitometry

Amounts of 1–5 μL were applied as bands or spots on the HPTLC plate (distance between spots 1 cm). The distance from the plate edge was 5 mm. Bandwise applications were done by the use of a CAMAG (Muttenz, Switzerland) Linomat III. For spot applications, calibrated micro-capillary pipets (CAMAG) were used. The separation was performed in a CAMAG horizontal developing chamber by use of the mobile phase acetonitrile–water (8 : 2). The amino plate was developed from both sides to a distance of 45 mm. The separation is accomplished within 8 min. Subsequently, the wet plate was heated for 20 min at 190°C in an oven. At this temperature, Sucralose® is able to react with the amino groups of the HPTLC layer and formed a brilliant fluorescent spot with an R_f value of 0.73. The spots were inspected under ultraviolet (UV) light (365 nm, 8 W mercury lamp) directly or after the dipping the developed plate for 5 sec in a cetyl-trimethylammonium bromide solution (5 g in 100 mL of methanol), which resulted in fluorescence enhancement by a factor of two.

The spots could also be measured densitometrically in the absorption mode. For direct spectrophotometry of the HPTLC plates, a diode-array scanner from J&M (Aalen, Germany) was used, which is able to measure TLC or HPTLC plates simultaneously at different wavelengths. This new TLC scanner consists of a diode array spectrophotometer (J&M Aalen, Germany) working in a range of 197–612 nm with an average optical resolution of better than 2.0 nm.[12] An attachment of 50 identical optical fibers transports light of different wavelengths from a deuterium lamp to the HPTLC plate and back to the DAD. In this detecting mode, light emitting fibers and light detecting fibers are both placed at a distance of 450 μm above the surface of the HPTLC plate.[12] For dense light intensities, the light emitting and the light detecting fibers are arranged parallel to each other, because in this arrangement a nearly vertical detection mode results. The HPTLC plate is placed horizontally on a mechanical stage, which can be moved by use of two motors from Micropack Company (Stuttgart, Germany). The linear slide system works with constant velocity during reflection measurements. The J&M SPECTRALIS software is used for instrumental control and data collection. The whole device does not need any lenses, filters, or slit-width adjustments.

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Measurements of absorption for Sucralose® determination were done in a spectral range from 270 to 330 nm. All single diodes (DAD) measuring in the chosen range are combined into one densitogram in order to increase the signal-to-noise ratio.[13] For measurements of the fluorescence spectra, as shown in Fig. 3, a UV source with a peak emission of around 370 nm is used to provide UV light into the glass fibers for plate illumination. The diode is purchased from Nichia (Nuernberg, Germany). The fluorescence emission was measured in a spectral range from 440 to 480 nm.

RESULTS AND DISCUSSION

Separation by Thin Layer Chromatography

An important advantage of planar chromatography is the use of disposable plates, which in contrast to other separation methods, such as HPLC, GC, CE, or IC, can often reduce the requirements for sample clean-up. The HPTLC method described here can be applied for the detection and determination of Sucralose® in various food matrices.

Sucralose® combines both lipophilic and hydrophilic groups in its molecular structure. It was difficult to predict its interaction with the HPTLC plates, and it was found to be rather difficult to obtain a satisfactory separation with different mobile phases commonly used for the separation of carbohydrates. Little changes in solvent strength caused a dramatic change in R_f values. For this reason, the use of a β-front separation for Sucralose® was decided upon, and it worked excellently for this application. A β-front is formed during the chromatographic process, because the amino-layer selectively absorbs water from the mobile phase. This results in a water free phase-system in front of the β-front. In this phase-system, all lipophilic substances are moving with the β-front. Hydrophilic compounds stay at the point of application or move only slightly forward. As a result, all other carbohydrates and intense sweeteners show R_f values less than 0.25. For example, saccharin moves with an R_f value of 0.40 and sodium benzoate shows an R_f value of 0.31. The Sucralose® spot is well separated and results in an R_f value of 0.73 (β-front). The separation of an undiluted "Cola" softdrink spiked with 48 mg/L of Sucralose® is shown in Fig. 2.

Normally, β-fronts in HPTLC separations are not favorable because normal chromatographic behavior is not seen within the very steep gradient of the β-front. For this reason, R_f values of substances that move in a β-front are not comparable to those moving outside the front. In addition, the peak width for a substance moving within a β-front is much smaller as a result of the steep gradient in the front. Thus, it can appear that several substances

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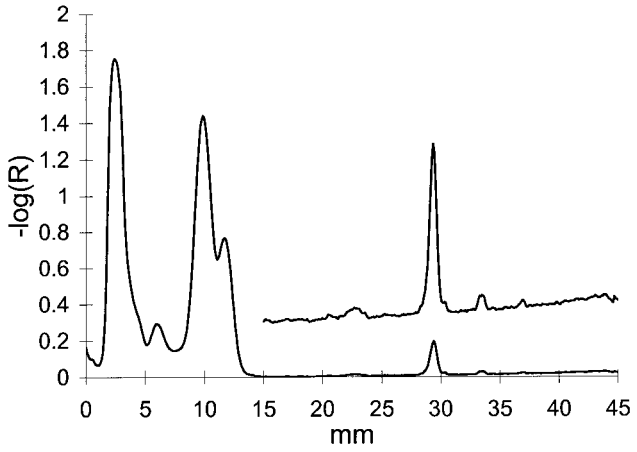

Figure 2. Densitogram of a "Cola" type softdrink in the absorption mode (1 μL spotted) containing 48 mg/L Sucralose® (peak at 29 mm). The upper signal line is plotted 5-fold enhanced.

moving together with a β-front are not separated and, therefore, appear as a single peak. However, a β-front separation can offer many advantages.[12] The formation of β-fronts is very reproducible on TLC plates, and this is responsible for a highly constant R_f value for Sucralose®. In addition, the very small peak width of the Sucralose® spot after separation further increases the detection limit, since the local analyte concentration is more highly condensed compared to substances that move outside the β-front. In our new method, this offered a detection limit of 5 ng of Sucralose® per spot in densitometric absorption measurements.

The separation was performed in horizontal developing and was accomplished within 8 min. Subsequently, the wet plate was heated for 20 min at 190°C in an oven. At this temperature, Sucralose® is able to react with the amino groups of the HPTLC layer and forms a brilliant fluorescent spot at an R_f value of 0.73.

Densitometry

The resulting fluorescent spots that are obtained during the heating process of the plate also show absorption properties that can be densitometrically measured in absorption mode as they appear as a yellow colored derivative when viewed in daylight. In situ densitometry offers a simple

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method of quantification by measuring the optical density of the separated spots directly on the plate.[13] During densitometric measurements, the illuminating light is either absorbed or scattered. Only the scattered light is reflected from the plate, and this light provides the desired information. The reflected light is called remission light.

Quantitative evaluation of thin-layer chromatograms by optical methods is based on a differential measurement of light emerging from the sample-free and sample-containing areas of the plate, although the relationship between detector response and sample concentration is not simple.[14–17] To a first approximation, a parallel light beam with the intensity I is used for illuminating the HPTLC plate. To get a lamp-independent spectrum, it is recommended to use the quotient of the sample spectrum and the spectrum of the clean HPTLC plate. Usually, the remission values are calculated from the measurement values of the HPTLC track, $I_{\text{rem}}(\lambda)$, divided by reference values $I_0(\lambda)$:

$$R(\lambda) = \frac{I_{\text{rem}}(\lambda)}{I_0(\lambda)} \tag{1}$$

A proper election of the reference spectrum $I_0(\lambda)$ is a very effective method for baseline corrections. A convenient way to transform remission data into mass-dependant signals is to use formula (2):

$$A(I_{\text{rem}}, \lambda) = -\ln R(\lambda) \tag{2}$$

The logarithm expression sets all intensities smaller than I_0 into positive values. If the spot emits more light than the reference, the sample shows fluorescence. The fluorescence spectrum is derived from sample spectrum and reference spectrum using Eq. (3):

$$F(I_{\text{rem}}, \lambda) = \frac{[I_{\text{rem}}(\lambda) - I_0(\lambda)]}{1000} \tag{3}$$

The resulting absorbing and fluorescent Sucralose® derivative shows a suitable absorption maxima around 300 nm and fluorescence around 450 nm. The absorption and fluorescence spectra are shown in Fig. 3. For quantification purposes both modi, UV-absorption and fluorescence, can be used.

For UV absorption analysis, a Sucralose® detection limit of 1 mg/L (5 μL applied on the plate) was achieved. A calibration curve in a range from 10 to 380 ng is shown in Fig. 4. Even though it was expected that fluorescence measurements should have been superior to UV absorption, it must be mentioned that illumination was provided with a low power LED (370 nm). This LED resulted only in a very low fluorescence intensity because its

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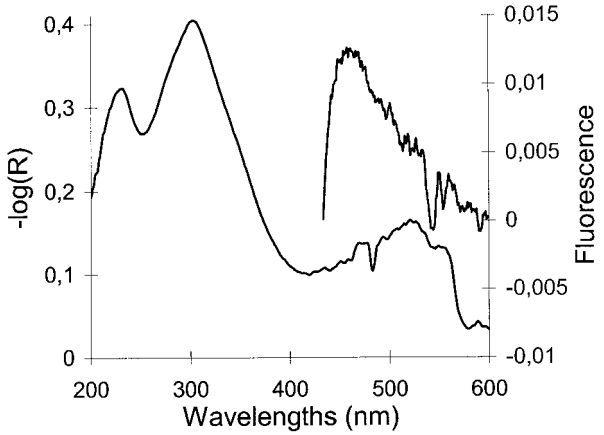

Figure 3. Absorption and fluorescence (upper graph) spectrum of the Sucralose® derivative on the amino HPTLC plates in the range of 200–600 nm.

maximum wavelength is far from the absorption maximum of the derivatized Sucralose® (Fig. 3).

The applied derivatization reaction, termed thermochemical activation, is well known for carbonyl compounds such as sugars separated on amino-bonded silica gel.[18–21] Mostly, an oven temperature of 150°C was not exceeded. Reactions of amino-HPTLC plates with sugars;[12,18] creatine, creatinine, and uric acid;[20] and catecholamines[22] are described in the literature.

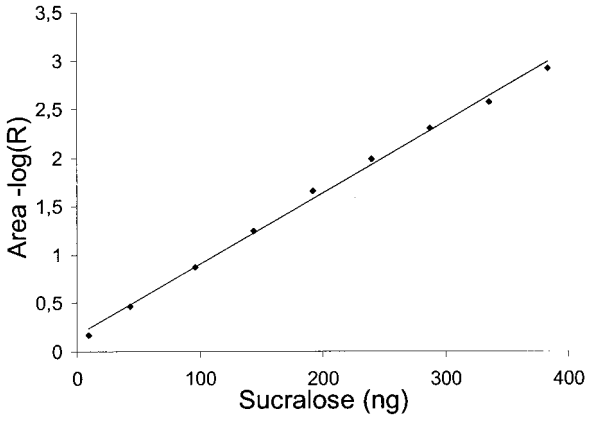

Figure 4. Linear relation (calibration graph) of Sucralose® in the range of 10–380 ng.

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All these substances produce a brilliant fluorescence on a non-fluorescent background, upon heating. It has been speculated that the substances undergo a Maillard reaction, losing water and forming high molecular mass derivatives.[19]

We report here for the first time the determination of a chloro-organic compound undergoing this reaction type to form a highly fluorescent derivative of an unknown structure, and it must be stressed that, different from former publications, a reaction temperature of 190°C was used to obtain the desired results, which is beyond the temperatures reported for other compounds.[19–22]

Method Performance Criteria

Several commercial soft drinks (diet and standard) from the European market were analyzed for Sucralose® in order to determine the method performance criteria in various beverages. As expected, Sucralose® was not found in any of the samples (it is not yet authorized for use). For repeatability, spiking of various beverages with Sucralose® resulted in a relative standard deviation of 3.4% ($n = 9$), with an average recovery rate of 95%. The high sample throughput and the very low analytical costs (consumables) of less than 40% in comparison to HPLC[22] makes the method ideal for food screening.

The detection limit for visual semi-quantification under UV light at 365 nm was found to be 30 mg/L of Sucralose® (5 μL of solution spotted). This could be improved by a factor of two by treating the developed plate with cetyltrimethylammonium bromide solution to achieve fluorescence enhancement.

Better quantification with uncertainties below 5% and detection limits of 1 mg/L of Sucralose® can be achieved by the use of a diode-array scanner. This makes the HPTLC method superior to all previously published methods. No interference with other intense sweeteners such as saccharin and aspartame; carbohydrates such as sucrose, fructose, and glucose; or preservatives such as sodium benzoate was observed.

CONCLUSION

The presented method for the determination of Sucralose® in food can be easily and rapidly performed. It does not require any chemical derivatization reagent and offers a convenient way to quantify Sucralose® without laborious pre-cleaning steps. A very stable β-front formed during the separation allowed an interference-free determination of Sucralose® in all food matrices tested, and is responsible for highly constant R_f values. The resulting small peak

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width offers additional advantages concerning the detection limit. These advantages justify the use of a β-front as a separation tool. The use of the diode-array scanner results in excellent detection limits and makes the presented method superior to all other published methods.

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