Rapid analysis of sucralose in various food matrices

Morlock, G., Vega, M., Schwack, W.

Institute of Food Chemistry, University of Hohenheim

Scope

Sucralose is a relatively new sweetener which has been approved for use in numerous food and pharmaceutical products in over 60 European countries since January 2005. Its importance is mainly based on three facts: 1) It is a free flowing, non-hygroscopic, relatively heat resistant powder which is convenient for use in food technology, 2) it has a taste profile very similar to sugar without any after taste, and 3) it shows a sweetening intensity of 600-650 times that of sucrose allowing its incorporation in food at very low levels.
Analysis of sucralose in foods is quite difficult because of the lack of a chromophore. Available RP-HPLC methods employ RID or UV absorption (≤ 200 nm), besides pre-column derivatization. The general trend to transfer such analyses to HPLC-MS or HPLC-ELSD thus leads to a general increase in analysis costs. Additionally, a solid phase extraction is necessary due to the matrix load intolerance and universal detection principle of the detectors used. The issue was addressed whether HPTLC can be a **low-cost alternative**.

Results and discussion

A new HPTLC method on silica gel plates has been established, with which 46 runs were performed with acetonitrile and water 17:3 (v/v) within 13 min [1]. This means, due to its intrinsic capability of parallel chromatography, **17 seconds per run**. Densitometry was performed either at 500 nm after post-chromatographic **derivatization** with 2-naphthol sulfuric acid reagent (brownish band for sucralose) or at 405 nm after post-chromatographic derivatization with aniline diphenylamine o-phosphoric acid reagent (gray-bluish band for sucralose). With the latter reagent a bluish fluorescence at 366/>400 nm was also observed for sucralose; this enabled a very sensitive and selective detection comparable with that on amino phases (Fig. 1).

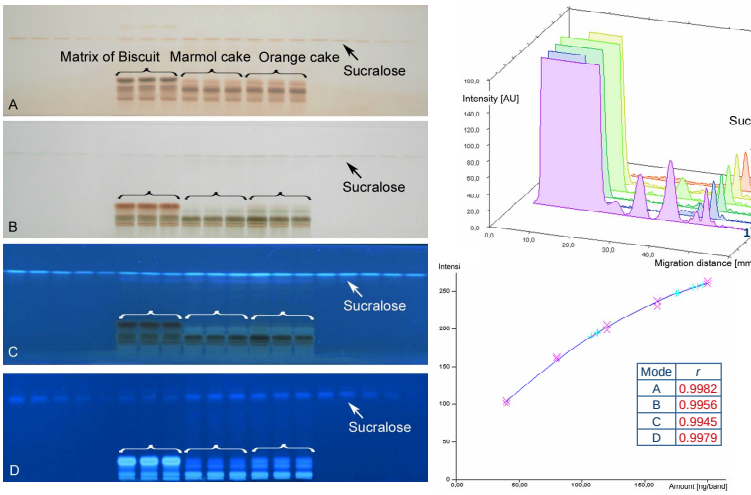
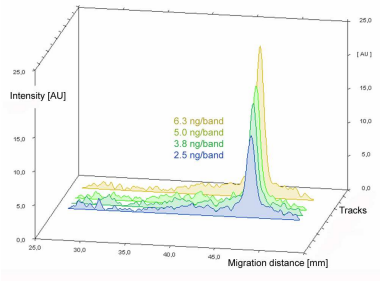
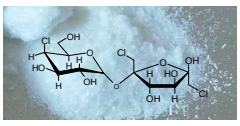


Fig. 1: ***Left*** **Documentation of plate images A) in the visible range after derivatization with 2-naphthol sulfuric acid reagent, B) in the visible range and C) under UV 366/>400 nm, both after derivatization with aniline diphenylamine o-phosphoric acid reagent, and D) under UV 366/>400 on amino phases just heated;** ***Right*** **Calibration exemplarily shown for detection mode A (r = 0.9982).**

The new methods based on silica gel phases were compared with the *reagent-free* method on amino phases [2] with regard to **limit of detection** (LOD) of sucralose (Fig. 2).





Mode	Measurement of	LOD (S/N 3)
A	Absorbance at 500 nm, reagent 1	5 ng/band
B	Absorbance at 405 nm, reagent 2	8 ng/band
C	Fluorescence at 366/400 nm, reagent 2	4 ng/band
D	Fluorescence at 366/400 nm, heated	6 ng/band

Fig. 2: ***Left*** **LOD of sucralose at 3.8 ng/band after derivatization with aniline diphenylamine o-phosphoric acid reagent followed by fluorescence measurement at UV 366/>400 nm (detection mode C);** ***Right*** **Comparison of the LODs obtained by the respective measurement modes.**

The sucralose content found in dietetic biscuits and cakes was shown to be **highly repeatable** (*RSDs* ≤ 4.4 %, n = 3) and reliable with regard to the target value of the sucralose labeled on the packages (Table 1). According to the target value t-test the means obtained were not significantly different from the labeled values.

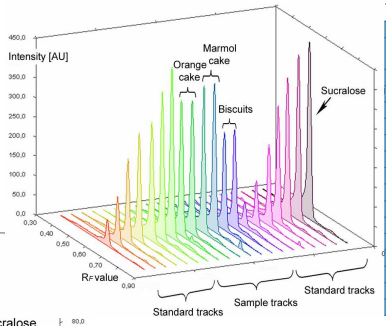


Table 1: Sucralose content found in dietetic bakery products

Samples	hR_F	Sucralose found (mg/100 g)	RSD (%) n = 3	Sucralose label (mg/100g)
Mode A: Reagent 1, measured at 500 nm				
Biscuits	57	27.7	2.4	24.8
Marmol cake	57	48.0	2.0	45.3
Orange cake	56	43.9	0.6	45.3
Mode B: Reagent 2, measured at 405 nm				
Biscuits	56	27.9	1.5	24.8
Marmol cake	56	47.4	0.5	45.3
Orange cake	56	44.2	1.6	45.3
Mode C: Reagent 2, measured at UV 366/>400 nm				
Biscuits	56	27.1	0.9	24.8
Marmol cake	57	44.8	4.2	45.3
Orange cake	56	41.6	3.0	45.3
Mode D: Amino phase heated, measured at UV 366/>400 nm				
Biscuits	55	22.2	2.2	24.8
Marmol cake	57	45.7	4.4	45.3
Orange cake	57	46.8	2.2	45.3

Fig. 3: ***Left up*** **Fluorescence measurement of sucralose at UV 366/>400 nm after derivatization with aniline diphenylamine o-phosphoric acid reagent (detection mode C); standards 10 to 200 ng/band;** ***Left*** **Separation of sucralose in (1) milk, (2) shortbread biscuit, (3) chocolate, (4) cola, (5) hard candies, (6) energy/sport drink, exemplarily shown for detection mode B.**

Selectivity for different matrices (Fig. 3) was achieved for all the detection modes used. Specificity was even obtained by use of the detection mode C because only sucralose produced a fluorescent color despite the presence of carbohydrates and other matrix constituents. All matrices were mainly fixed at low hR_F values enabling a very **reduced sample preparation**, i.e. just dissolution/suspension of the sample and filtration.

Conclusion

Good accuracy despite high matrix load HPTLC is well suited to quantification of sucralose not only in energy/sport drinks [3] and milk-based confectionery [2], but also in dietetic cakes, biscuits and various other matrices. All in all, 46 chromatographic runs can be performed within 40 min. Sample throughput is by a factor of 3 higher, however, because a single HPTLC working place can be used in a step-stacked system of 15-min intervals meaning while plate 1 is applied, plate 2 is developed and plate 3 detected. In an 8-hour shift approximately **1000 samples** can be quantified by a single HPTLC working place. In reality, however, the factor limiting sample throughput is **sample preparation.** Fortunately this can be kept simple when HPTLC is used.

Thanks to CAMAG and Merck for material support granted.

[1] G. Morlock, M. Vega, J Planar Chromatogr 20 (2007) 411-417
[2] G. Morlock, S. Prabha, J. Agric. Food Chem. 55 (2007) 7217-7223
[3] Private communication, Interlaboratory trial 2007, JRC, Geel, Belgium