



Rhodamine-Sulphuric Acid -A New Visualization Reagent for the Determination of Sucralose by HPTLC

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Abstract: Sucralose a UV-visible inactive compound was separated on silica gel plate without any plate treatment prior to analysis, derivatized with rhodamine - sulphuric acid reagent and detected densitometrically at 456 nm as olive green band. With this reagent sucralose also shows golden yellow fluorescence at 366 nm. Two new solvent systems *i.e.* chloroform: methanol: toluene (v/v 5:3.5:1.5) (solvent system-I) and chloroform: ethanol: benzene (v/v 5:3:2) (solvent system-II) were developed and giving R_f values of 0.62 and 0.45 respectively. The method was found to be sensitive with good limit of detection (LOD) for two solvent systems. The method imparts specificity to the method as at 456 nm sucralose only gives olive green color spots where as other artificial sweeteners did not show any response to this reagent, where as carbohydrates gives black color spots. Similarly sucralose gives golden yellow fluorescence at 366 nm which is not given by any other artificial sweetener. The method was highly reproducible with relative standard deviation (RSD) $\leq 3\%$ (n=3) and was applied for the determination of sucralose in different matrices like cola drinks, lemon juices, sugar free sweets, tabletop sweeteners *etc.*

Key words: Sucralose, Rhodamine-sulphuric acid, HPTLC, Beverages, Sweets.

Introduction

Sucralose, 1,6-dichloro-1,6-dideoxy- β -D-fructofuranosyl-(2 \rightarrow 1)-4-chloro-4-deoxy- α -D-galactopyranoside) (Figure 1) is made by substituting 3 hydroxyl groups of sucrose with

chlorine atoms. Sucralose is approximately 600 times as sweet as sucrose. It is stable under heat and over a broad range of pH concentration and can be used in baking or in products that require a longer shelf life. Sucralose does not promote tooth decay. The European Union's scientific committee on food, U.S food and drug administration (FDA) approved its use in 1998. The acceptable daily intake is in general has been agreed to be 15 mg/kg/day. It also has been approved by the regulatory agencies of many countries for use in numerous food and pharmaceutical preparations.

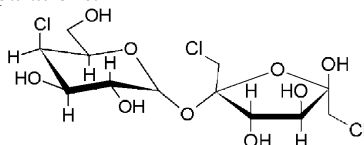


Figure 1. Sucralose structure

Sucralose was analyzed by RP-HPLC methods employing refractive index detection¹⁻³, pre-column derivatized HPLC by treatment with *p*-nitrobenzoyl chloride (PNBCL)⁴, simultaneous determination with other non-nutritive sweeteners in foods by HPLC/ESI-MS⁵, HPLC-ELSD⁶ capillary electrophoresis⁷ and isotope dilution LC/MS/MS⁸. All these methods are tedious and require complicated sample preparation and are expensive. Compared to all these methods planar chromatographic methods offer simple, fast, simultaneous multi sample handling and cost effective method. Sucralose was detected by reagent free derivatization on amino-phase TLC plates⁹⁻¹¹, even though these methods are simple but the amino-phase TLC plates are three to four time costly than normal silica gel coated plates and also for derivatization we are suppose to heat the plate for 20 minutes at 190 °C. Sucralose was separated also on silica gel coated TLC plates and detected by derivatization with aniline diphenylamine orthophosphoric acid / naphthol sulfuric acid¹². However these methods require pretreatment of plates by impregnation with dipotassium hydrogen phosphate solution (0.1 M).

This paper describes a simple method for separation of sucralose on silica gel plate without any plate pretreatment and detected by post derivatization by rhodamine-sulfuric acid in visible as well as fluorescence mode with good sensitivity.

Experimental

Sucralose was supplied by Virchows laboratory (Hyderabad, India), where as sucrose and dextrose was purchased from Qualigens (Mumbai, India). All solvents used were of analytical grade obtained from S.d. fine chemicals (Mumbai, India). Ultra pure water was produced by a synergy system (Millipore, Schwalbach, Germany). Cola drinks, lemon juices, tabletop sweeteners and dietetic sweets were purchased from local market. Rhodamine 6G (Basic Red 1), sulphuric acid and ethanol were purchased from Merck, India.

Apparatus

For the chromatographic investigations, Camag, Switzerland equipment comprising of a TLC Scanner II with a computer system and winCats Software V. 1.4.2, ATS4 application device, a twin-trough chamber, a TLC Immersion Device III, Camag TLC Plate heater were used. Aluminum HPTLC plates coated with silica gel F₂₅₄ (20 cm × 10 cm) were supplied by Merck (Germany).

Procedure

Commercially available tabletop sweeteners and dietetic sweets shows the sucralose content as 70 mg/g and 15 mg/10 g respectively. The samples were ground and a representative aliquot 1 g of dietetic sweets and 10 mg of tabletop sweetener respectively were transferred

to 10 mL volumetric flasks and suspended in methanol made up till the mark while shaking. Extraction of sucralose was performed in an ultrasonic bath for 20 min. Samples were filtered through a Whatmann filter paper No.4 before application to the stationary phase.

Commercially available cola drinks, lemon juices, sweets did not contain sucralose and were used for spiking with sucralose (cola drinks and lemon juices were spiked at 100 mg L⁻¹, 80 mg L⁻¹, 60 mg L⁻¹ and sweets were spiked at 1 g kg⁻¹, 1.5 g kg⁻¹ & 2 g kg⁻¹). Cola drinks and lemon juices (1 mL each) were diluted 1:1 with methanol where as sweets were homogenized and 1 g was extracted with 10 mL of methanol. Cola drinks and lemon juices were ultrasonicated for 10 min where as sweets samples were ultrasonicated for 20 min and filtered through a Whatmann filter paper No.4 before application to the HPTLC plate. For calibration, a 0.1 mg mL⁻¹ working solution of sucralose in methanol was prepared.

Application

For determination the solutions were applied to the plates as 4 mm bands by spraying with the Automatic TLC Sampler 4 (ATS4), enabling a maximum of 21 tracks to be applied to one 20 cm x 10 cm HPTLC plate (distance from lower edge 15 mm, distance from the left side 10 mm, distance between bands 9 mm). For calibration, 0.5-2.5 µL of the standard solution (50-250 ng per band) was applied to the plate five times. The dietetic sample solutions (tabletop sweetener and dietetic sweets) were applied as 1 µL and 2.5 µL respectively. For determination of limit of detection 0.1 to 1 µL of the 10 ng µL⁻¹ standard solution was applied to the plate. Whereas 1 µL of spiked samples of cola drinks, lemon juices and sweets were applied to the plate for recovery studies.

Chromatography

Aluminum plates were developed in a 20 cm x 10 cm twin-trough chamber (TTC). Silica gel plates were developed with the mobile phase, Chloroform: methanol: toluene (5:3.5:1.5) (solvent system-1) and chloroform: ethanol: benzene (5:3:2) (solvent system-2). The development distance was 75 mm from the application point and the migration time was approximately 20 min for both solvent systems.

Derivatization

Rhodamine-sulfuric acid solution was prepared by adding 3 mL of sulfuric acid to 20 mL of 0.25% ethanolic solution of rhodamine with continuous stirring. The reagent was stable for at least for two hours at room temperature.

Post-chromatographic derivatization of sucralose on silica gel was performed with the TLC Immersion Device III using a dipping speed of 3 cm s⁻¹ and dipping time of 1 s, followed by heating of the plate on the TLC plate heater III at 120 °C for 3 min. Sucralose derivative was visible as olive green spots where as carbohydrates appeared as black spots against light yellow background. With the reagent sucralose and carbohydrates gave spots of golden color and red fluorescence at 366 nm respectively. The fluorescence spots were visualized at 366 nm (reflectance mode) in the Digitostore 2 documentation system consisting of the reprostar 3 illuminator and the powershot G2 digital camera (Canon, Tokyo, Japan).

Evaluation and documentation

Densitometric evaluation with the TLC Scanner 3 was performed via peak area by fluorescence measurement at 366 nm and by absorbance measurement at 456 nm in the calibration range 50 to 250 ng sucralose / band. Plate images were documented by use of the Digitostore2 documented system under illumination at 366 nm and in the visible range, all in the reflectance mode. The data obtained were processed with winCATS software, version 1.4.2.

Results and Discussion

Performing selective derivatization of sucralose on the plate is a strong feature of planar chromatography because all chromatographic runs were derivatized simultaneously followed, usually by a few minutes of heating. Sucralose was separated on silica gel 60 F₂₅₄ HPTLC plates without any impregnation of TLC plates prior to analysis using chloroform: methanol : toluene (5:3.5:1.5) and chloroform : ethanol : benzene (5:3:2) by volume giving R_f values of 0.45 and 0.62 respectively. Sucrose, dextrose and other matrices did not interfere with the analysis of sucralose despite the simple sample preparation. This method can be used selectively for analysis of sucralose in the presence of other artificial sweeteners. The reagent did not form any derivative with other artificial sweeteners like saccharin, aspartame and cyclamate. A great advantage of the proposed HPTLC method is simple sample preparation unlike the use of a solid phase extraction step used for HPLC-MS-MS⁸ or HPLC-ELSD⁶.

Visualization was enabled densitometrically by post-chromatographic derivatization of sucralose with rhodamine-sulfuric acid reagent (Figure 2). Sucralose gives olive green spot (Figure 3A) and its identity was confirmed by recording visible spectrum (Figure 4). With this reagent sucralose also gives golden fluorescence at 366 nm where as carbohydrates gives red fluorescence (Figure 3B). The optimum wavelength for absorbance measurement in the visible range was found to be 456 nm. Figure 5 shows a typical calibration curve for sucralose after derivatization with rhodamine-sulfuric acid and absorbance measurement at 456 nm.

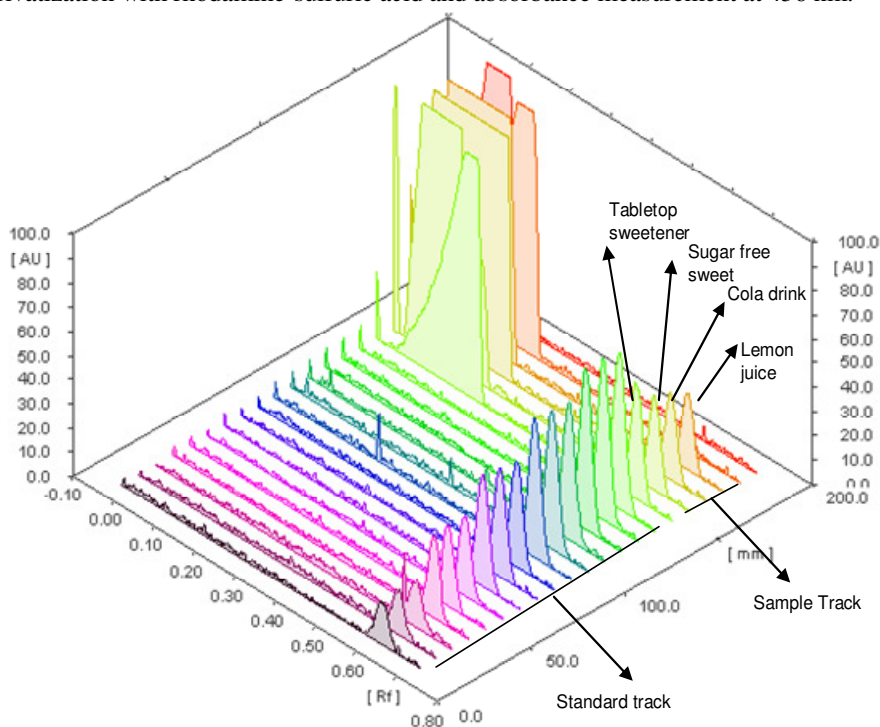


Figure 2. Absorbance measurement of sucralose at 456 nm after derivatization with the rhodamine-sulfuric acid reagent; tracks 1-15 sucralose standards tracks 16, 17, 18, 19 are of Cola drink, Lemon juice, Sugar free sweet and Tabletop sweetener respectively (Solvent system-I)

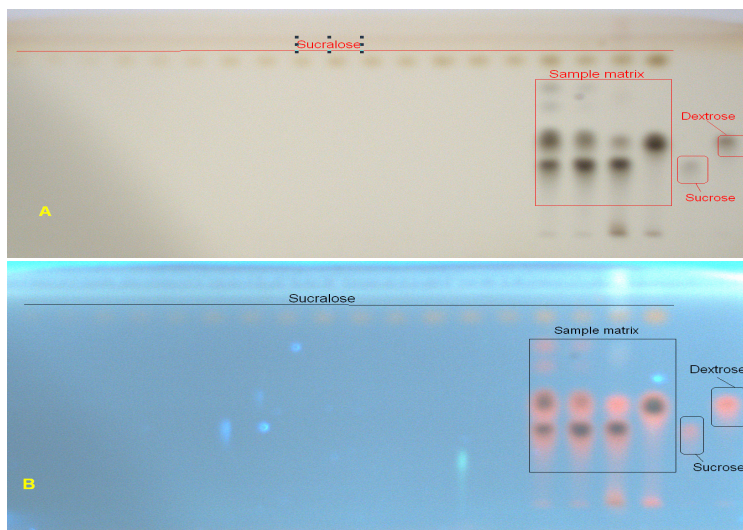


Figure 3. Documentation of plate images (illuminated in reflectance mode) obtained during quantification of sucralose. (A) 456 nm (B) 366 nm after derivatization with rhodamine-sulfuric acid (Solvent system-I)

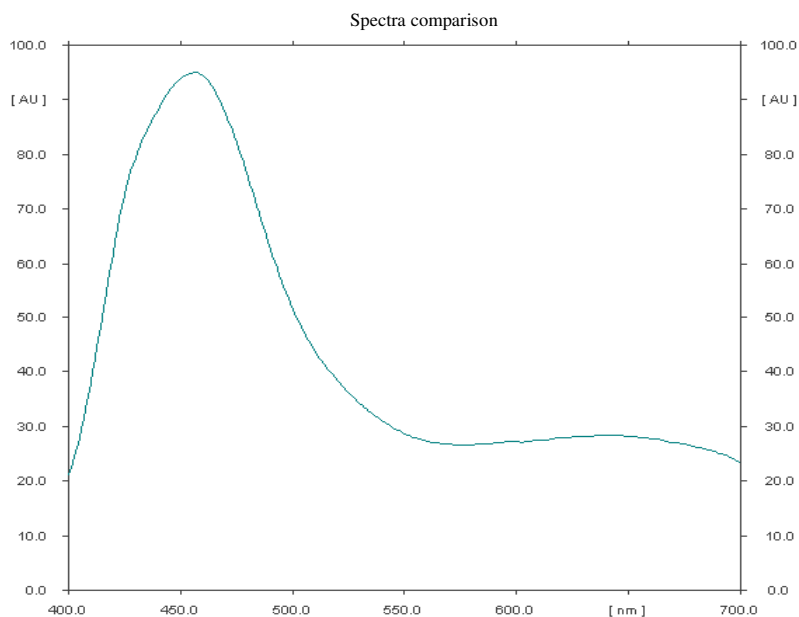


Figure 4. Absorbance spectrum of sucralose–rhodamine complex showing an absorbance maximum at 456 nm (Solvent system-I)

In absorbance mode at 456 nm LOD for this method was found to be 5 - 7 ng where as in fluorescence mode at 366 nm it was found to be 4 - 5 ng. LOD for the two solvent systems was found to differ marginally. The method was found to be linear in the concentration range of 40 - 250 ng with the correlation coefficients, r^2 were better than 0.9881 (Table 1).

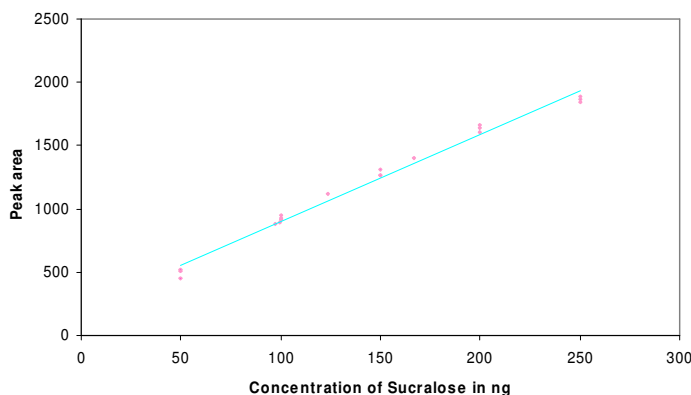


Figure 5. Linear calibration of sucralose derivatized with rhodamine-sulfuric acid reagent ($y = 6.0143x + 244.35$) by peak area of absorbance measurement at 456 nm in the range between 50 and 250 ng per band with relative standard deviation of the calibration plot $\pm 2.3\%$ and correlation coefficient $R^2=0.9947$ (Solvent system-I).

Table 1. Comparison of the calibration data obtained for sucralose using two solvent systems after derivatization in different modes of measurement.

Solvent System	Measurement mode	Regression equation	R^2
I	Absorbance at 456 nm	$y = 6.0143x + 244.35$	0.9947
II	Absorbance at 456 nm	$y = 6.9029x + 207.58$	0.9875
I	Fluorescence at 366 nm	$y = 6.8169x + 224.21$	0.9864
II	Fluorescence at 366 nm	$y = 6.8255x + 227.27$	0.9881

The recovery data in cola drinks, lemon juices and sweets spiked with sucralose for solvent system-1 and solvent system-2 at absorbance 456 nm and at fluorescence UV 366 nm shown (Table 2) to be better with relative standard deviation (RSD) less than 3 (n=3). Where as data for commercially available dietetic samples were shown in Table 3.

Table 2. Data for recovery of sucralose in spiked samples obtained using two solvent systems after derivatization with rhodamine-sulfuric acid reagent in absorbance mode at 456 nm and in fluorescence mode at 366 nm.

Sample	Recovery%			
	Solvent system 1		Solvent system 2	
	Measurement at 456 nm	Measurement at 366 nm	Measurement at 456 nm	Measurement at 366 nm
(i) Sweet samples spiked with sucralose				
1 g kg ⁻¹	96	96.34	95.76	96.12
1.5 g kg ⁻¹	94.21	95.01	94.09	94.59
2 g kg ⁻¹	98.22	98.76	97.70	97.93
(ii) Cola drinks spiked with sucralose				
100 mg L ⁻¹	98.27	99.57	98.32	99.41
80 mg L ⁻¹	98.12	98.79	96.51	97.01
60 mg L ⁻¹	98.76	99.10	96.98	97.32
(iii) Lemon juices spiked with sucralose				
100 mg L ⁻¹	97.05	98.21	96.21	97.95
80 mg L ⁻¹	98.32	99.12	97.77	98.09
60 mg L ⁻¹	96.10	96.98	95.98	96.21

Table 3. Data for recovery of sucralose in real samples obtained using two solvent systems after derivatization with rhodamine-sulfuric acid reagent in absorbance mode at 456 nm and in fluorescence mode at 366 nm.

Sample	hR _f	Amount of Sucralose found, mg per 1 g	RSD% (n=3)	Label declaration, mg per 1 g
Mode: Measurement of Absorbance at 456 nm. Solvent System-I				
Sugar free sweet	62	1.24	2.5	1.5
Tabletop sweetener	61	65.9	1.9	70
Solvent System-II				
Sugar free sweet	45	1.235	2.3	1.5
Tabletop sweetener	45	65.4	1.5	70
Mode: Measurement of Fluorescence at 366 nm Solvent System-I				
Sugar free sweet	62	1.247	2.1	1.5
Tabletop sweetener	61	66.4	2.0	70
Solvent System-II				
Sugar free sweet	45	1.26	2.12	1.5
Tabletop sweetener	45	68.3	2.08	70

Conclusion

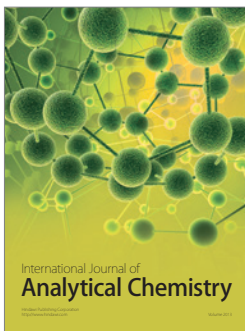
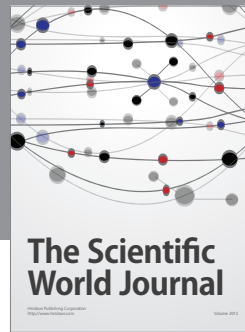
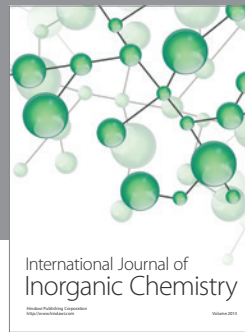
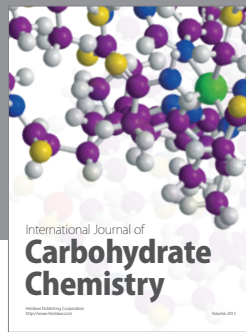
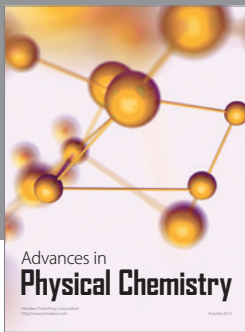
The method proposed for the determination of sucralose in tabletop sweeteners, cola drinks, lemon juices and sugar free sweets is simple, fast, sensitive, specific, reliable and selective when compared to the existing methods. Due to its capability of simultaneously handling multiple samples it can be adapted for use in pharmaceutical and food industries. Because of its high through put the method can also be used for routine work in food, drug, forensic and environmental regulatory laboratories.

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