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Simultaneous Determination of Saccharin and Aspartame in Commercial Noncaloric Sweeteners Using the PLS-2 Multivariate Calibration Method and Validation by Capillary Electrophoresis

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A new method to determine a mixture for sweetener sodium saccharin and aspartame in commercial noncaloric sweeteners is proposed. A classical full factorial design for standards was used in the calibration step to build the partial least-squares (PLS-2) model. Instrumental data were obtained by means of UV−visible spectrophotometry. Salicylic acid was used as an internal standard to evaluate the adjustment of the real samples to the PLS model. The concentration of analytes in the commercial samples was evaluated using the obtained model by UV spectral data. The PLS-2 method was validated by capillary zone electrophoresis (CZE), finding in all cases a relative error of less than 11% between the PLS-2 and the CZE methods. The proposed procedure was applied successfully to the determination of saccharin and aspartame in noncaloric commercial sweeteners.

KEYWORDS: Sweeteners; sodium saccharin; aspartame; PLS-2 method; multivariate calibration; capillary electrophoresis

INTRODUCTION

Saccharin (1,2-benzisothiazolin-3-one-1,1-dioxide) is the oldest and one of the strongest sweeteners on the market, possessing about 300 times the sweetening capacity of sugar. Sodium saccharin, which is often used because it dissolves better than saccharin, is still 450 times sweeter than sugar.

The acceptable daily intake (ADI) for saccharin was increased to 5.0 mg kg^{-1} body weight by the Joint Expert Committee on Food Additives (JECFA) in 1993. The Scientific Committee on Food of the European Commission (SCFEC) increased the ADI for saccharin to 5.0 mg kg^{-1} body weight in 1995 (*1, 2*).

Aspartame (*N*-L-α-aspartyl-L-phenylalanine methyl ester) is 200 times sweeter than sugar. Used all over the world in foods and beverages, it contains two amino acids, aspartic acid and phenylalanine. Studies in a number of animal species indicate that aspartame is quickly and extensively metabolized to its constituent amino acids and methanol. According to experimental model systems, aspartame is reported to have low toxicity. The ADI for aspartame has been set at 40 mg kg^{-1} body weight by the JECFA and at 40 mg kg^{-1} body weight by the SCFEC in 2000 (*1, 3*).

A great variety of methods have been applied to the analysis of the aforementioned compounds in foods, soft drinks, dietary products, and pharmaceutical preparations; however, there are no suitable procedures for the simultaneous determination of sodium saccharin and aspartame by use of a combination of partial least-squares (PLS) and UV−visible spectrophotometry. Nowadays, high-performance liquid chromatography (HPLC) is the most frequently used technique (*4*−*8*). In addition, ion chromatography (IC) offers an attractive alternative to traditional HPLC methods (*9*−*11*). In the past few years, micellar electrokinetic chromatography (MEKC) and capillary zone electrophoresis (CZE) have been applied to the simultaneous determination of several kinds of sweeteners (*12*−*18*). Other methods less commonly used for saccharin and aspartame determination are FT-Raman spectroscopy (*19*), FT-Raman spectroscopy with chemometric analysis (*20, 21*), amperometry based on the use of bilayer lipid membranes (*22*), spectrophotometry based on the complexation of aspartame with Cu (*23*), a biosensor for aspartame determination (*24*), the use of an ion selective electrode for saccharin determination (*25, 26*), and sequential flow injection coupled with enzymic detection (*27, 28*).

However, all of these methods are based on expensive analytical instruments. For this reason, in this paper, a new, fast, and inexpensive method for the simultaneous determination

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of two artificial sweeteners, aspartame and sodium saccharin, by using the PLS-2 multivariate calibration and UV−vis spectrophotometry, is proposed. The recovery degree and PLS model fit in samples were evaluated by use of salicylic acid as an internal standard. The results obtained in real samples of commercial sweeteners were validated by CZE. This method is useful for the determination of saccharin and aspartame in commercial noncaloric sweeteners.

PLS regression is an important multivariate calibration tool based on the use of a large number of variables, which permits one to evaluate the concentration of interesting analytes (*29, 30*). It can be used in two ways: PLS-1 calculates the concentration of one analyte per model, while PLS-2 can determine all analytes in a unique model. The PLS method is a full spectrum multivariate calibration tool that has been growing in importance for the last years and has been incorporated in new analytical chemistry textbooks (*31, 32*).

MATERIALS AND METHODS

Instrumental and Reagents. Spectrophotometric measurements were taken using an Ocean Optics model CHEMUSB4 UV−vis spectrophotometer with a linear charged-coupled device (CCD) array detector (Duiven, The Netherlands). pH measurements were taken with a pH meter HORIBA F42 (Tokyo, Japan). The CZE data were obtained by a Beckman P/ACE MDQ instrument (Beckman Instruments, Inc. Fullerton, CA) equipped with a diode array detector and a data handling system comprised of an IBM personal computer and P/ACE SYSTEM MDQ software. The fused silica capillaries were obtained from MicroSolv Technology Corp. (New Jersey) and had the following dimensions: 64.5 cm total length, 56 cm effective length, and 50 μm i.d. The PLS-2 data analysis was carried out using the Unscrumbler 6.11 software (CAMO ASA, Trondheim, Norway).

The water used in all studies was ultrapure water (18.2 MΩ cm) obtained from a Barnstead Easy pure RF compact ultrapure water system (Dubuque, IA). Sodium saccharin and aspartame, chloride and salicylic acids, sodium hydroxide, and potassium chloride were purchased from Sigma (St. Louis, MO). Sodium tetraborate was purchased from Mallinckrodt (St. Louis, MO). All other reagents and solvents were of analytical grade quality. All solutions (for the CZE method) were degassed by a Testlab ultrasonicator (Buenos Aires, Argentina) and filtered through a 0.45 μm Millipore filter (Billerica, MA).

Preparation of Real Samples. For the PLS-2 method, five commercial noncaloric sweeteners were analyzed. For liquid sweeteners, 1 mL of each sweetener was transferred into a 200 mL volumetric flask and diluted with ultrapure water. For table top sweeteners, 0.0850 g of sweetener was accurately weighed (±0.0001 g) and directly dissolved in ultrapure water and transferred into a 100 mL volumetric flask. For the CZE method, samples were prepared by diluting adequate volumes of previously mentioned sweetener solutions with ultrapure water.

CZE Reference Procedure. The CZE operation parameters, similar to those stated by Frazier and Cols (*18*), were as follows: The detection was performed at 210 nm, the capillary temperature was maintained at 25 °C, and the voltage was set at 20 kV. Samples were pressure-injected at the anodic side in the hydrodynamic mode at 25 kPa s. During sample analysis, a constant voltage and temperature were applied. The background electrolyte (BGE) used consisted of a 20 mM sodium tetraborate buffer, pH 9.4 (*18*). Aspartame and saccharin stock solutions were prepared in both cases, as 0.5 g L^{-1} in 250 mL volumetric flasks and diluted to the mark with ultrapure water. Six standard solutions and five replicates of each one were prepared by diluting adequate volumes of stock solution with concentrations of 5.0×10^{-4}, 1.0×10^{-3}, 1.5×10^{-3}, 3.0×10^{-3}, 2.5×10^{-3}, and 5.0×10^{-3} (g L^{-1}) for aspartame and 5.0×10^{-3}, 1.0×10^{-2}, 1.5×10^{-2}, 3.0×10^{-2}, 2.5×10^{-2}, and 5.0×10^{-2} (g L^{-1}) for saccharin; r^2 coefficient values for both the aspartame and the saccharine calibration plots were 0.996 and 0.998, respectively. The repeatability of the CZE system was evaluated performing successive injection ($n = 10$) of 3.0×10^{-3} and 3.0×10^{-2} g L^{-1} for both aspartame and saccharin, respectively, in combined solution. The relative standard deviation (RSD) values were better than 0.3% for the time of migration and 2.4% for peak area.

Table 1. Concentration Matrix for the PLS-2 Model

standard	aspartame[a]	saccharin[a]	internal standard[a]
1	0.000	0.000	0.000
2	0.001	0.000	0.002
3	0.003	0.000	0.008
4	0.005	0.000	0.000
5	0.000	0.010	0.004
6	0.001	0.010	0.002
7	0.003	0.010	0.008
8	0.005	0.010	0.000
9	0.000	0.030	0.008
10	0.001	0.030	0.002
11	0.003	0.030	0.004
12	0.005	0.030	0.004
13	0.000	0.050	0.008
14	0.001	0.050	0.004
15	0.003	0.050	0.002
16	0.005	0.050	0.000

[a] Concentration expressed in gL^{-1}.

PLS Model. Aspartame, saccharin, and salicylic acid stock solutions were prepared as 0.5, 0.5, and 0.25 g L^{-1} respectively, into 250 mL volumetric flasks and diluted to the mark with ultrapure water. The buffer solution of pH 2 was prepared from chloride acid and potassium chloride (*33*); a suitable amount of stock solution (mixture of sweeteners and internal standard) was transferred into a 25 mL volumetric flask; 5.0 mL of chloride acid buffer solution, pH 2, was added and diluted to the mark with ultrapure water.

The model was obtained using a total of 16 standard solutions mixtures of aspartame and saccharin and adding different levels of internal standard to each one, in concentrations similar to those of the analytes. A full factorial design was used to build the calibration matrix, with four levels and two variables. Spectrophotometrical readings were carried out on different days to bring more robustness to the PLS-2 model and to produce minor error levels in the prediction step. This is an important feature due to the use of complete full-spectra data, which are affected by instrumental variations, producing little changes in absorbance values and significant levels of noise (*34*). The concentration matrix used in the calibration step is shown in **Table 1**. All standard solutions were read from 200 to 330 nm every 1 nm with a cell of 10 mm optical path, obtaining a unique model, useful for all samples.

The PLS-2 model was made using the Unscrumbler 6.11 software tools. The calibration step was performed by the combination of the response matrix (R) 16 × 131 (16 standard solutions × 131 wavelength absorbance values) and the concentration matrix (C) of 16 × 2 (16 standard solutions × 2 analytes). The model was obtained using autoscaled data.

RESULTS AND DISCUSSION

Figure 1 shows the spectral overlapping of aspartame, saccharin, and internal standard in a 200−330 nm range. **Table 2** shows the explained variance (cumulative percentage) obtained in the calibration and validation processes with the PLS-2 method. Four PLS principal components (PCs) were needed in the calibration sets to explain 98.0, 99.9, and 99.3% of the original information for aspartame, saccharine, and internal standard, respectively. This parameter is useful to explain variances from original R and C matrices in the PLS-2 model as a function of the PLS PCs (*30*). The obtained r^2 coefficients in the observed predicted concentration plot for aspartame, saccharin, and internal standard in the PLS-2 model were 0.997, 0.999, and 0.998, respectively, suggesting a good fit in the model. The linear range for this method went from 0.01 to 0.05 g L^{-1} for saccharin and from 0.001 to 0.005 g L^{-1} for aspartame. A minor or major concentration of this range produces, for the

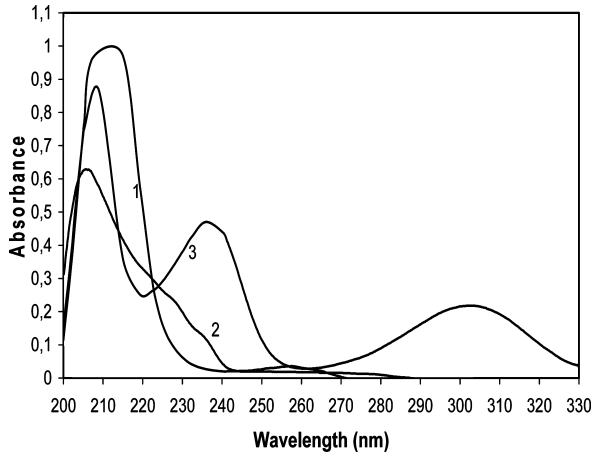

Figure 1. Spectral curves of aspartame 1 (0.005 g L^{-1}), saccharin 2 (0.005 g L^{-1}), and internal standard 3 (0.008 g L^{-1}).

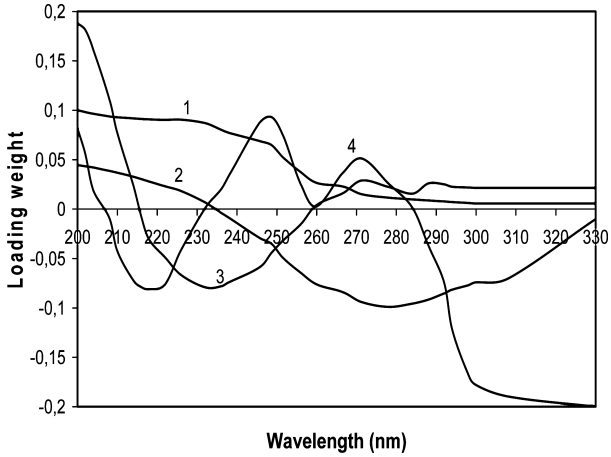

Figure 2. Loading weights as function wavelength for first (1), second (2), third (3), and fourth (4) PLS PCs.

Table 2. Percentage of Explained Variance for Aspartame, Saccharin, and Internal Standard in the Calibration and Validation Model Set

	aspartame		saccharin		internal standard	
PC	calibration	validation	calibration	validation	calibration	validation
0	0	0	0	0	0	0
1	−6.8	−17.7	94.1	93.0	6.2	−1.2
2	−2.0	−21.7	99.8	99.7	99.1	99.0
3	93.6	92.1	99.8	99.8	99.0	98.9
4	98.8	98.0	99.9	99.9	99.6	99.3

analytes and internal standard, a diminution of r^2 coefficient values in the observed−predicted concentration plot and a loss of fit in the PLS model.

Figure 2 shows the loading weight plot for the PLS PCs behavior, as function of wavelength, and it describes the influence of wavelength for each PC, also representing their relative contribution to the model (*30*). In this figure, the first PLS PC has a maximum in the range from 200 to 250 nm, coinciding with the maximum of aspartame (210 nm), saccharin (226 nm), and internal standard (208 and 237 nm) for the first and second maximum spectra. The second component presents a minimum at 278 nm, which coincides with the second maximum of saccharin spectra. The third component presents a minimum at 237 nm, coinciding with the second maximum of internal standard spectra. The fourth component presents a minimum at 219 nm, coinciding with an inflection wavelength of internal standard.

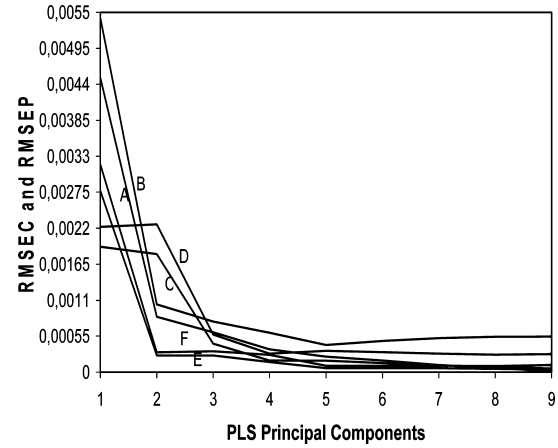

Figure 3. RMSEC and RMSEP for saccharin, aspartame, and internal standard. Saccharin: A, calibration; and B, validation. Aspartame: C, calibration; and D, validation. Internal standard: E, calibration; and F, validation.

Table 3. Added−Predicted Concentrations of Internal Standard in the Sample Set to Evaluate Matrix Effect and Recovery Degree

	internal standard			
simple	added[a]	predicted[a,b]	recovery (%)[b]	% Er[c]
liquid 1	0.0040	0.0039 ± 0.0002	97.5 ± 5.2	−2.5
liquid 2	0.0010	0.0011 ± 0.0003	110.0 ± 8.1	10.0
liquid 3	0.0080	0.0079 ± 0.0002	98.8 ± 2.6	−1.3
table top 1	0.0060	0.0059 ± 0.0002	98.3 ± 3.8	−1.7
table top 2	0.0080	0.0081 ± 0.0001	101.3 ± 3.0	1.3

[a] Concentrations expressed in g L^{-1}. [b] Average ± standard deviation of five determinations. [c] Percent relative error of recovery.

Model Internal Validation. The model was built using, in the first place, internal validation (cross-validation method), where the model leaves out one standard of the calibration set. This standard was used to predict and find the internal error of the model. When all standards were left out once, the calibration and validation model error could be calculated through root-mean-square of error calibration (RMSEC) and root-mean-square of error prediction (RMSEP) (*30, 31*). **Figure 3** shows the RMSEC and RMSEP of the PLS-2 model for aspartame, saccharin, and internal standard, which together with the % of explained variance helps to decide the adequate number of PLS PCs, by election of minimal value, which corresponds to the fourth PC in most cases, except the validation for saccharine, which had a minimum in the fifth PC.

Evaluation of the Predictive Ability of the Model. The predictive ability of the model was studied by means of an internal standard, which allows evaluation of the behavior of real samples and the recovery percentage in the model. This constitutes a very important matter to be able to know the predictive ability of the model in unknown samples, without external validation. If the internal standard added to samples shows a good fit in the PLS model, the same will occur with the analytes. This indicates that matrix effects in samples do not affect the PLS model. Salicylic acid was used as an internal standard, added to samples at random concentrations in levels similar to interesting analytes. **Table 3** shows the results of added and predicted concentrations of the internal standard in real samples using five replicates of each one: liquid 1 (Semblé), liquid 2 (Chuker), liquid 3 (Hileret), table top 1 (Sucaryl), and table top 2 (Chuker). In all cases, the predictive ability of the internal standard in real samples and using the proposed model has a mean value of 101%, and the percent relative error of the

Table 4. Predicted Concentrations in Real Samples by the PLS-2 Model and Validation by the CZE Method

	aspartame		saccharin	
sample	PLS-2[c]	CZE[c]	PLS-2[c]	CZE[c]
liquid 1[a]	1.5 ± 0.1	1.41 ± 0.02	27.4 ± 0.2	30.37 ± 0.07
liquid 2[a]	1.5 ± 0.1	1.39 ± 0.02	26.8 ± 0.2	29.81 ± 0.06
liquid 3[a]	5.0 ± 0.7	5.62 ± 0.03	26.8 ± 0.3	24.73 ± 0.07
table top 1[b]	37.7 ± 0.4	39.56 ± 0.08	99.2 ± 0.4	95.15 ± 0.11
table top 2[b]	36.9 ± 0.2	41.45 ± 0.07	96.8 ± 0.4	99.68 ± 0.20

[a] Concentrations in undiluted sweeteners expressed in g L^{-1}. [b] Concentrations in table top sweeteners expressed in mg g^{-1}. [c] Average ± standard deviation of five determinations.

model was not higher than 10%, which indicates that, due to dilution, the matrix effects do not alter the predictive ability of the model and the final results, obtaining an excellent recovery degree in all samples.

Real Samples Prediction and CZE Validation Results. Five samples of commercial noncaloric sweeteners obtained directly from the Argentine market were analyzed with five replicates by the PLS-2 model. Liquid samples were liquid 1 (Semblé), liquid 2 (Chuker), and liquid 3 (Hileret), and solid samples were table top 1 (Sucaryl) and table top 2 (Chuker). Results were validated by the CZE method. The results obtained by the PLS-2 and CZE methods are shown in **Table 4**. The relative error between both methods was less than 11% in all cases. This value is similar to that obtained by the PLS method in previous works for other types of samples (*34, 35*). The PLS and CZE reference methods were statistically compared (*36*).The *F* test was used for every sample to compare variances, obtaining *F* values from 1 to 5, which do not exceed the theoretical value of the 95% confidence level (6.388). The *t* test was used to compare means of every sample, obtaining *t* values from 0.3 to 1.9, lower than the theoretical value of the 95% confidence level (2.31).

Analytical Application. The results obtained show that the spectrophotometric methods combined with the PLS-2 data analysis permit the simultaneous determination of saccharine and aspartame in artificial commercial noncaloric sweeteners. The proposed method can be used without previous chemical separations and is simple, rapid, low-cost, and does not involve tedious manipulation steps. For these reasons, it is useful for the determination of aspartame and saccharin in commercial noncaloric sweeteners, offering fast and accurate results and becoming an alternative procedure for laboratories to routine analysis and food control.

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