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Analytical Methods

Simultaneous determination of aspartame and acesulfame-K by molecular absorption spectrophotometry using multivariate calibration and validation by high performance liquid chromatography

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ABSTRACT

A new method to determine mixtures of two sweeteners, aspartame and acesulfame-K, in commercial sweeteners is proposed. A classical 5^2 full factorial design for standards was used for calibration in the concentration matrix. Salicylic acid was used as internal standard in order to evaluate the adjustment of the real samples in the PLS-2 model. This model was obtained from UV spectral data, validated by internal cross-validation and was used to find the concentration of analytes in the commercial samples. The PLS-2 method was validated externally by high performance liquid chromatography (HPLC), finding, in all cases, a relative error of less than 10% between the PLS-2 and the HPLC methods. The mean value of recovery degree in real samples was 99.2% with standard deviation of 3.2%. The proposed procedure was applied successfully to the determination of mixtures of aspartame and acesulfame-K in artificial sweeteners.

1. Introduction

The presence in the market of non caloric sugar substitutes has increased because of the demand for new products, which can be used for management diabetic and dietetic reasons as well as in the manufacture of "tooth-friendly" food and pharmaceutical products (Parke, Birch, Portmann, & Kilcast, 1999). Therefore, good analytical methods to assure quality control and product integrity are essential in meeting the needs of this growing market.

Acesulfame-K (3,4-dihydro-6-methyl-1,2,3-oxathiazine-4-one-2,2-dioxide) is a high-intensity and non caloric sweetener. It is not metabolized by the body and is excreted unchanged. Acesulfame-K is currently used in food, beverage, oral hygiene, and pharmaceutical products in about 90 countries; it has about 200 times the sweetening capacity of sugar. The acceptable daily intake (ADI) for acesulfame-K was increased in 1991 to 15 mg kg^{-1} body weight by both, the Joint Expert Committee on Food Additives (JECFA) and the Scientific Committee on Food of the European Commission (SCFEC) (International Sweeteners Association, 2004; Von Rymon Lipinsky, 1991).

Aspartame (N-L-a-aspartyl-L-phenylalanine methyl ester) is between 160 and 220 times sweeter than sugar. It contains two amino acids, aspartic acid and phenylalanine. Studies in a number of animal species indicate that aspartame is quickly and extensively metabolized in its constituent amino acids and methanol. Experimental model systems show that aspartame has low toxicity. In 2000, ADI for aspartame has been set at 40 mg kg^{-1} body weight by both, the JECFA and SCFEC (Homler, Deis, & Shazer, 1991; International Sweeteners Association, 2004; US Department of Health and Human Services, Public Health Service, National Institute of Health, 2003). In recent years, aspartame was studied because it was objected as sweetener and food additive. The safety of aspartame and its metabolic constituents was established through extensive toxicology studies in laboratory animals, using much greater doses than people could possibly consume. Nonetheless, additional research, including evaluations of possible associations between aspartame and headaches, seizures, behaviour, cognition, and mood as well as allergic-type reactions and use by potentially sensitive subpopulations, has continued after approval in 1980. It is clear that aspartame is safe, and there are not unresolved questions regarding its safety under conditions of intended use (Butchko et al., 2002).

A great variety of methods have been applied to the analysis of the aforementioned compounds in foods, but only a few procedures are suitable for the simultaneous determination of aspartame and acesulfame-K in commercial sweeteners. High performance liquid chromatography (HPLC) is the most frequently used technique nowadays (Dossi, Toniolo, Susmel, Pizzariello, &

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Bontempelli, 2006; Kobayashi et al., 1999; Tsang, Clarke, & Parrish 1985; Wasik, McCourt, & Buchgraber, 2007; Wu, Cheng, & Chou, 1995). In addition, ion chromatography (IC) offers an attractive alternative to traditional HPLC methods (Chen & Wang, 2001; Zhu, Guo, Ye, & James, 2005). 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 in foods (Boyce, 1999; Frazier, Inns, Dossi, Ames, & Nursten, 2000; Pesek & Matyska, 1997).

Other methods less commonly used for aspartame and acesulfame-K determination are FT-Raman spectroscopy (Armenta, Garrigues, & De La Guardia, 2004), FT-Raman spectroscopy with chemometric analysis (Khurana, Cho, Shim, Li, & Jun, 2008), amperometric method based on the use of bilayer lipid membranes (Nikolelis & Pantoulias, 2000) spectrophotometry based on the complexation with Cu (Fatibello-Filho, Marcolino-Junior, & Pereira, 1999), biosensors for aspartame determination (Odaci, Timur, & Telefoncu, 2004), sequential flow injection coupled with enzymic detection (Pena, Lima, Saraiva, & Lucia, 2004) and flow injection sensor with on-line solid-phase extraction (Vallvey, Valencia, Nicolás, & García-Jiménez, 2006).

The instrumental methods above mentioned are important referential methods, but are based on expensive analytical instruments, which are not available for laboratories of routine in quality control and low resources. On this basis, a new, fast and inexpensive method for the simultaneous determination of mixtures of aspartame and acesulfame-K using the partial least square (PLS-2) multivariate calibration, is proposed in this work. The quality of analytical results obtained in five real samples of commercial sweeteners by PLS-2, was validated by high performance liquid chromatography (HPLC) method. The behaviour of the model in the real samples was evaluated using salicylic acids as internal standard, which was incorporated in the PLS-2 model.

The partial least square (PLS) regression method is a multivariate calibration tool that was used in a large number of previous works to determine the concentration of several analytes in a wide variety of samples, in combination with different spectroscopic techniques (Brereton, 2000; Galeano Diaz, Guiberteau, Ortiz Burguillos, & Salinas, 1997; Lozano, Camiña, Boeris, & Marchesvky, 2007; Ni, Zhang, & Kokot, 2005). PLS uses the original spectroscopic variables, which allows to evaluate the concentration of mixtures of analytes (Beebe, Pell, & Seasholtz, 1998; Massart et al., 1997). The PLS-2 method is a multivariate calibration tool that uses a unique model to predict simultaneously the concentration of analytes in real samples (Martens & Naes, 1996).

2. Experimental

2.1. Instrumental

UV spectral measurements were taken using an Ocean Optics model CHEMUSB4 UV–vis spectrophotometer with linear CCD array detector (Duiven, The Netherlands). The spectrophotometric measures were carried out at room temperature (about a mean of 20 °C). pH measurements were taken with a pH metre HORIBA F42 (Tokio, Japan). The HPLC data were obtained by KONIK KNK-500-A Series (Miami, FL, USA). A 25 cm C-18 column Lichrosorb RP18 (USA) was used with KONIK UV detector (Miami, FL, USA).

The PLS-2 data analysis was carried out using the Unscrumbler 6.11 software (CAMO ASA, Trondheim, Norway).

2.2. Reagents

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, IO, USA). Acesulfame-K and aspartame, chloride and salicylic acids, potassium dihydrogen phosphate and potassium chloride, were purchased from Sigma (St. Louis, MO, USA). Acetonitrile HPLC grade was obtained from Merck (Durmsted, Germany).

2.3. Standard solutions

For the PLS-2 method, aspartame, acesulfame-K and salicylic acid stock solutions were prepared weighing accurately 0.5000, 0.5000 and 0.2500 g respectively, dissolving and diluted to the mark whit ultrapure water into a 1000 ml volumetric flasks. The buffer solution of pH 2.0 was prepared using chloride acid and potassium chloride, following pH values by Clarck and Lubs (Meites, 1963). The concentration matrix for calibration step was obtained with a suitable amount of stock solution (mixture of sweeteners and internal standard) and transferred to a 25 ml volumetric flask. Five millilitres of chloride acid buffer solution pH 2.0 was added and diluted to the mark with ultrapure water. The absorbance of these mixtures was measured in 10 mm quartz cells between 200 and 330 nm at 0.2 nm intervals with respect to a blank of chloride acid buffer solution pH 2.0.

For HPLC method, aspartamo and acesulfame-K stock solutions were prepared weighing accurately 0.1000 g l^{-1} for both cases. The mobile phase was prepared with 10% (v/v) acetonitrile and 90% (v/v) 0.02 mol l^{-1} potassium dihydrogen phosphate solution. Standard solutions were obtained diluting adequate volumes of aspartame and acesulfame-K stock solutions with a mobile phase into 10 ml volumetric flasks.

2.4. Real samples preparation

For the PLS-2 method, five solid (powder) commercial sweeteners were prepared weighing (0.8000 ± 0.0001) g of sweeteners, which were directly dissolved in ultrapure water and transferred into 100 ml volumetric flasks. From this solution, 2 ml were transferred to 250 ml volumetric flasks and added with a buffer solution pH 2 to mark. For the HPLC method, samples were prepared diluting adequate volumes of the above mentioned sweeteners solutions with a mobile phase: 10% (v/v) acetonitrile and 90% (v/v) 0.02 mol l^{-1} potassium dihydrogen phosphate solution. These sample solutions were filtered before injection.

2.5. HPLC procedure

Seven standard solutions and five replicates were prepared for both, aspartame and acesulfame-K, with concentrations of 3.0×10^{-3}, 4.0×10^{-3}, 5.0×10^{-3}, 6.0×10^{-3}, 7.0×10^{-3}, 8.0×10^{-3} and 9.0×10^{-3} (g l^{-1}). The HPLC conditions were: carrier flow 0.75 ml min^{-1}, absorbance measure using a single wavelength at 205 nm, similar to those stated by (Armenta et al., 2004). The obtained calibration curves yielded a R^2 regression coefficient of 0.9992 and 0.9988 for aspartame and acesulfame-K respectively, and were used to validate the PLS-2 method. The repeatability of HPLC system was evaluated performing successive injection ($n = 6$) 5.0×10^{-3} g l^{-1}, for aspartame and saccharin in combined standard solution. The Relative Standard Deviation (RSD) values were better than 0.3% for aspartame and 0.1% for acesulfame-K.

3. Results and discussion

3.1. The PLS model

A full factorial design 5^2 was used to build the calibration matrix, with five levels of concentration and two variables. The PLS-