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Analytical Methods

Quantification of aspartame in commercial sweeteners by FT-Raman spectroscopy

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ABSTRACT

Efficient methods are proposed herein for the quantification of aspartame in commercial sweeteners. These methods are based on a treatment of Raman data with partial least squares (PLS), principal component regression (PCR) and counter-propagation artificial neural networks (CP-ANN) methods. For the three chemometric techniques used, the relative standard errors of prediction (RSEP) calculated for calibration and validation data sets were on the order of 1.8–2.2%. Four commercial preparations containing between 17% and 36% of aspartame by weight were evaluated by applying the developed models. Concentrations found from the Raman data analysis agree perfectly with the results of the UV–Vis reference analysis, with the recoveries in the 98.7–100.8%, 98.6–101.1% and 97.8–102.2% ranges for the PLS, PCR and CP-ANN models, respectively. The proposed procedures can be used for routine quality control during the production of commercial aspartame sweeteners.

1. Introduction

Aspartame, N-L-α-aspartyl-L-phenylalanine methyl ester, is an artificial sweetener widely used in the food, beverage, confectionery and pharmaceutical industries. It is a white, crystalline powder with a sweetness potency that is about 200 times that of sucrose. The substance is present in a vast number of mainly dietary products, and its annual consumption is counted in millions of kilograms.

Several methods have been developed for aspartame quantification in food products, with high performance liquid chromatography (HPLC) being the most popular method (Di Pietra, Carvini, Bonazzi, & Benfenati, 1990; Gibbs, Alli, & Mulligan, 1996; Tyler, 1984; Verzella, Bagnasco, & Mangia, 1985; Wasik, McCourt, & Buchgraber, 2007; Webb & Beckman, 1984). However, there are also examples of the use of capillary electrophoresis (Herrmannovà, Křivànkova, Bartoš, & Vytřas, 2005; Pesek & Matyska, 1997), flow injection (Capitán-Vallvey, Valencia, Nicolás, & García-Jiménez, 2006; Peña, Limba, & Saraiva, 2004), biosensors (Campanella, Aturki, Sammartino, & Tomassetti, 1995) and voltammetry (Medeiros, De Carvalho, Rocha-Filho, & Fatibello-Filho, 2008) applications for aspartame quantitative determination. Spectrophotometry (Cantarelli, Pellerano, Marchevsky, & Camiña, 2009) and infrared spectroscopy (Khurana, Cho, Shim, Li, & Jun, 2008; Kizil, Irudayaraj, & Seetharaman, 2002) in conjunction with multivariate techniques of data treatment were applied for aspartame quantification. The use of surface enhanced Raman scattering (SERS) for its identification has also been reported (Peica, 2009). The diversity of the forms of the commercial products containing this sweetener has prompted a continuous search for new, effective methods for its rapid quantification.

Raman spectroscopy has become an increasingly important tool in the quantitative analysis of complex mixtures (Pelletier, 2003; Strachan, Rades, Gordon, & Rantanen, 2007). It is a non-destructive technique that yields reliable results for solid and liquid multicomponent samples, particularly when the analyte concentration exceeds 0.5–1.0% by weight. Unlike many analytical techniques, this method usually does not require the dissolution or extraction of the samples being analysed, substantially simplifying and shortening the analysis. In addition, after recording Raman spectra, one can then perform additional analyses of the same sample using other analytical tools. Another benefit of the method, especially in comparison with infrared (IR) spectroscopy, is the fact that the presence of water does not hinder the analysis of liquid samples. Additionally, the Raman spectrum is often less complex than the IR spectrum, which simplifies the analysis. The unique advantage that the Raman technique offers is the fact that the analysed substance can be quantified in its original package, i.e. in a polymer blister, in a bottle, or in an ampoule. In industrial applications, its high capacity for automation is also very important. As such, Raman spectroscopy is a convenient tool for the quantitative analysis of multicomponent systems and is applied in forensic science (Ryder, O'Connor, & Glynn, 2000), pharmaceuticals (De Beer et al., 2007; King, Mann, & Vickers, 1985; Mazurek & Szostak, 2009; Skoulika & Georgiou, 2001) and food products analysis (Armenta, Garrigues, & de la Guardia, 2004; Peica, 2009).

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The successful quantification of an analyte in the sample on the basis of Raman data relies on a number of factors. The first is connected to the stability of conditions during measurements. Changes of the spectrometer and environmental parameters and poor repeatability result in a lack of correlation between the intensity changes in the recorded spectra and the sample composition. This effect can be eliminated, at least to certain extent, by appropriate normalisation and pre-processing (Bowie, Chase, & Griffiths, 2000a; Bowie, Chase, & Griffiths, 2000b; Szostak & Mazurek, 2009). The second factor influencing the quality of analyte determination is the method of analysis. In the classical univariate approach, a calibration curve is first constructed. To make this procedure possible, it is often necessary to extract the analysed compound or to find spectral regions where only undistorted features of the analyte are present. In the Raman analysis of complex mixtures, it is not always possible to neglect the bands of the additives, especially in the case of strongly overlapping spectra or spectra of low quality. This is extremely important when an analysed substance is a weak Raman scatterer. In such a case, only multivariate techniques, such as PLS, PCR or ANN, offer the possibility of reliable quantification.

For suitable modelling of the studied system, proper experimental design and preparation of mixtures is essential. The mixtures should contain, if possible, all of the substances present in the analysed product whose concentrations change, and the mixtures should contain these substances in the concentration ranges one might encounter in the analysed samples. In fact, even the complete qualitative composition of a commercial product is often not known to the analyst. Although it is possible to perform the qualitative analysis, some additives present in small amounts are not always detected. An important limitation of this type of analysis is the fact that the method is strictly valid only for products that are very similar to the calibration samples. If the composition of the calibration samples differs noticeably from that of the product, the analysis is much more laborious and can give unreliable results. When, however, the exact composition of the analysed sample is known, one can quantify its different constituents simultaneously on the basis of one calibration procedure. The easiest way to monitor the composition conformity of the calibration and commercial samples is to perform a principal component analysis (PCA) of the experimental data, in our case the Raman spectra, obtained for both categories of samples.

Herein, we present the procedure for aspartame quantification in four commercial tabletop sweeteners, in the form of tablets, by FT-Raman spectroscopy using the PLS, PCR and CP-ANN modelling methods.

2. Experimental

2.1. Instrumental

A Nicolet Magna 860 FT-IR spectrometer interfaced with an FT-Raman accessory and equipped with a CaF_2 beam-splitter and InGaAs detector was used to perform the measurements. The samples were placed in a rotating sample holder and were illuminated by an Nd:YVO_4 laser line at 1.064 μm with a power of 350 mW, without a converging lens. Backscattered radiation was collected at the sample. Samples were rotated at a constant speed of 200 rpm. The interferograms were averaged over 256 scans, Happ-Genzel apodised and Fourier transformed using a zero filling factor of 2 to give spectra in the 100–3700 cm^{-1} range at a resolution of 8 cm^{-1}.

UV–Vis spectra of aspartame solutions in the 200–330 nm range were recorded using a Carry 50 spectrometer.

HPLC measurements were performed at an industrial laboratory on a BioTek liquid chromatograph equipped with a UV/VIS 535 detector. Aspartame samples were analysed on a 5 μm LichroCART column (LichroSpher 100, 250–4 mm) using a mobile phase of phosphate buffer + methanol (70:30, v/v) at the flow rate of 1 ml min^{-1} with UV detection at 200 nm.

2.2. Chemicals

Aspartame, carboxymethylcellulose sodium salt (CMCNa) and leucine were of analytical grade (Sigma), and lactose and magnesium stearate were of pharmacopoeial purity. Aqueous solutions for UV–Vis analysis were prepared using purified water (Millipore) that was characterised by a resistivity of 18 MΩ cm. Four aspartame tabletop sweeteners, containing approximately 15–20 mg of aspartame per tablet were purchased at a local store.

2.3. Sample preparation

Calibration and validation samples were prepared by mixing pure, solid substances in a mortar for 5 min to homogenise the powders properly. Suitable weight ratios of compounds were chosen such that the collinearity between the concentrations of the components was minimised. No significant correlations were observed, as the determination coefficient (R^2) value for aspartame versus additive concentrations changed in the 0.01–0.18 range. Approximately 200 mg of powder was used to prepare a pellet in a way similar to that adopted for IR spectroscopy. The commercial tablets were first ground and then processed in the same way as the calibration samples.

Samples for UV–Vis analysis were prepared according to the procedure described by Cantarelli et al. (2009).

2.4. Software and numerical data treatment

Nicolet TQ Analyst ver. 7 chemometric software was used to construct PLS and PCR models. The neural network simulations were performed with the help of software developed by Zupan, Novič, and Ruisánchez (1997). The numerical data were prepared and transformed into an appropriate format using the MATLAB environment. All spectral data were mean centred.

To characterise the predictive ability of the developed models and to compare them, the relative standard errors of prediction (RSEP) were calculated according to the equation:

$$\mathrm{RSEP}(\%) = \sqrt{\frac{\sum_{i=1}^{n}(C_i - C_i^A)^2}{\sum_{i=1}^{n} C_i^{A^2}}} \times 100, \quad (1)$$

in which C_i^A is the actual i-component content, C_i is the concentration found from Raman data analysis, and n is the number of samples. The $RSEP_{cal}$ and $RSEP_{val}$ parameters were determined for the calibration and validation data sets, respectively.

The cross-validation technique (leave-one-out) was performed for internal validation of the constructed models, and the R_{CV} correlation coefficient of the validation was computed to estimate the robustness of the models. The predicted residual error sum of squares (PRESS) was calculated to select an optimal number of factors for the PLS models.

2.5. Computational methods

The most popular chemometric tools used for quantitative modelling of the multidimensional spectroscopic data are the partial least squares (PLS) regression and the principal component regression (PCR) methods (Gabrielsson, Lindberg, & Lundstedt, 2002; Næs, Isaksson, Fearn, & Davies, 2002; Wold, Sjöström, &