HORIBA Scientific

Raman Spectroscopy

Quality control of sugar content in beverages using Raman spectroscopy

Application Note

Agri-food RA88

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Abstract: While almost all of us have consumed sugary drinks at least once in our lives, excessive consumption can be common due to the addictive nature of the high sugar content. This can lead to increased risk of health problems such as abdominal obesity and type 2 diabetes (1). To avoid some of the health risks of sugar, it can be replaced by sweeteners such as aspartame, one of the first synthetically produced, or naturally derived compounds like stevia. Added sweeteners such as these have lower sweetness than natural sugars. Whether it is sugar or sweetener, their content must be monitored for human consumption. Here, we demonstrate that Raman spectroscopy is an effective solution to identify and evaluate sugar/sweetener concentrations in quality control processes.

Keywords: Raman spectroscopy, sugars, trehalose, sucrose, maltose, lactose, stevia, aspartame, beverages, MVA plus multivariate analysis, MultiWell application.

Introduction

Sugar content of beverages final products like juices, sodas or ice teas is particularly controlled as there is a globally important consumption of sugary drinks that is not without any consequence on the health of world population. Overconsumption of sugary drinks is related to abdominal obesity and to a risk increase to develop type 2 diabetes. Content determination of sugar, as well as of its substitutes, so called sweeteners such as the “chemical” aspartame or the “natural” stevia, is of interest in final products as well as during different stages of beverages and food products production chains.

In this application note, we show how Raman spectroscopy is used to identify and determine sugar/sweetener concentrations of solutions using MVAPlus multivariate analysis application. Moreover, we demonstrate how this is performed in an automated routine analysis combined to the MultiWell application.

Materials and methods

The XploRA™ PLUS enables Raman chemical identification directly to the microscope, combining the full functionality of a microscope and high-performance Raman spectroscopy. Raman spectroscopy is based on the inelastic scattering that occurs during the interaction of light with the chemical bonds within a material. The easy-to-use XploRA™ PLUS system is ideal for research and analytical laboratories.

The XploRA™ PLUS equipped with a 532 nm laser, a 1800 g/mm grating and a 20x LWD objective was used for all studied sugars except for the aspartame. The aspartame analysis was performed under the following configurations:

- Tablet mapping was acquired using a 785 nm laser, a 1200 g/mm grating and a 50x LWD objective.
- Raman polarisation study on the tablet was acquired using a 638 nm laser, a 600 g/mm grating and a 50x LWD objective.
- Analysis of solutions was carried out with a 638nm laser, a 1200 g/mm grating and a 20x LWD objective.

Standard sugar solutions of four disaccharides: sucrose (glucose-fructose), trehalose (two non-reducing glucose molecules), maltose (two reducing glucose molecules) and lactose (glucose-galactose) were prepared in deionized water at different concentrations (0, 0.3, 0.5, 0.75, 1.125,

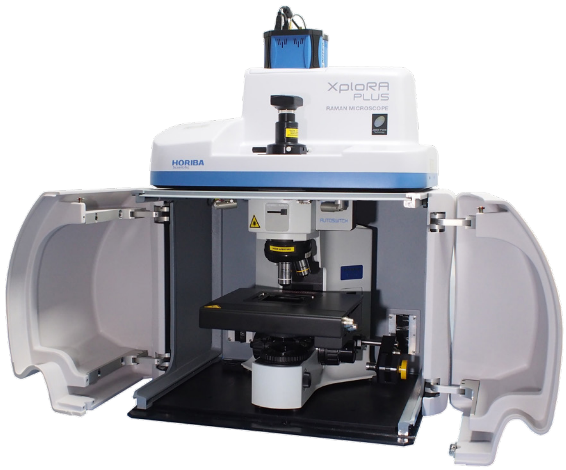

Figure 1: XploRA™ PLUS system and LabSpec 6 application used (MultiWell)

1.7, 2.55 and 3.825% wt/v). Standard solutions of two sweeteners were also prepared in deionized water at different concentrations: stevia at 0, 1.36, 2.05, 3.07 and 4.60% wt/v and aspartame at 0, 0.89, 1.34, 2.01, 3.01 and 4.52% wt/v. Two different types of common sugary beverages, citrus soda and orange juice, were also analyzed. No sample preparation was required for the commercial beverages (only a two- and three-times dilution in deionized water, respectively).

Additionally, for the standard sugar solutions, 8 different concentrations were prepared for each sugar and each standard was deposited in triplicate to ensure measurement repeatability, leading to a total of 48 samples where a 96-microwell plate was used for the Raman measurements. Using the LabSpec 6 MultiWell acquisition module, the XploRA™ PLUS system is completely compatible with automated measurements thanks to automated movement of the large sample stage and autofocus systems. Moreover, each well of the microplate was filled with a sample volume of 200 µL allowing Raman measurements with a low volume of sample.

Results and discussion

Raman imaging and polarisation study of an aspartame tablet

A 300 x 290 µm Raman map of an aspartame tablet was acquired over 13.5 hours (Figure 2, Left). The obtained Raman map was analyzed using multivariate analysis based on a Classical Least Squares (CLS) method. The CLS method uses a supervized algorithm: reference spectra (Figure 2, Right) with acesulfame K in red, aspartame in blue and lactose in orange were acquired and used for the analysis. Each compound is assigned a color and this is attributed to each point measured based on the decomposition of reference spectra, generating the Raman image. Identification of reference spectra is confirmed by the Raman KnowItAll® database provided with LabSpec 6 software and powered by Wiley Science Solutions. Following this, for one point of the map having a main contribution of aspartame, a Raman polarization study was performed. Polarization of the incident laser beam and of the Raman signal was varied either vertically or horizontally , normal to the sample plane. Spectra obtained for the different combinations are compared in Figure 3 (150-1800 cm^{-1}

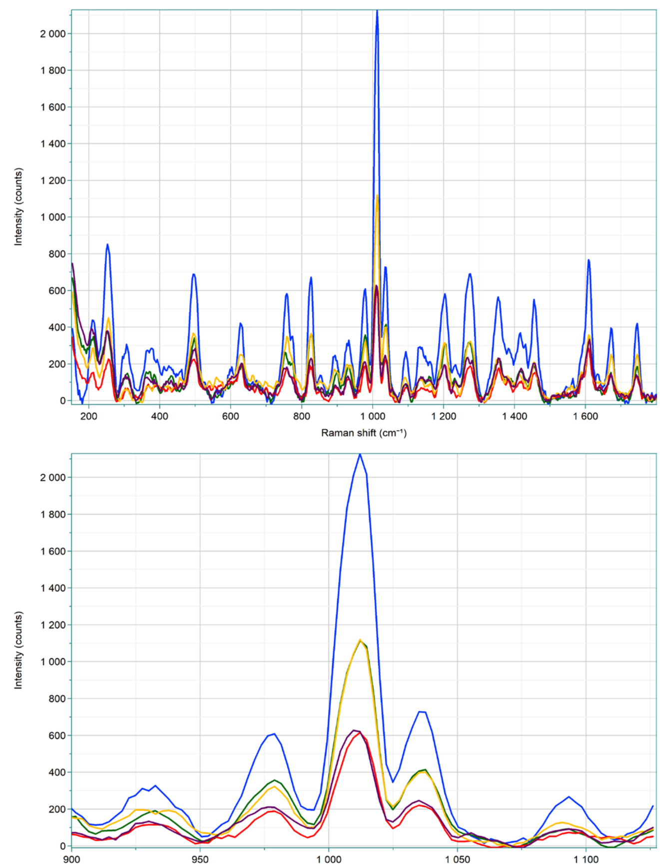

Figure 3: Top) Overlay of spectra obtained at a point of aspartame of the tablet after variations of the polarisation of the incident laser beam and of the Raman signal; Bottom) Zoom-in on the 900-1125 cm^{-1} range.

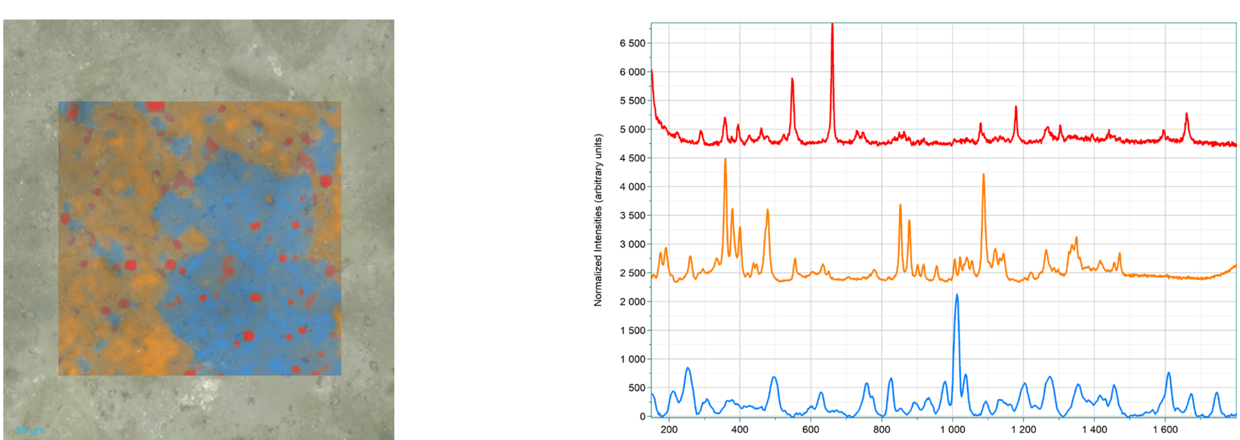

*Figure 2: Left) Overlay of an aspartame tablet video image with its corresponding Raman image generated using multivariate analysis (components distribution); Right) Reference spectra for multivariate analysis (CLS method). **Red:** acesulfame K, **Blue:** aspartame and **Orange:** lactose.*

range on the top and zoom-in on the 900-1125 cm^{-1} range on the bottom). Focussing on the 900-1125 cm^{-1} range, especially on the highest band at 1010 cm^{-1}, we can observe that the intensities decrease for Y-X and X-Y configurations compared to Y-Y and X-X configuration. Raman polarization is particularly useful in differentiating enantiomers, which are present in sugar molecules where different enantiomers have individual fingerprints.

Standard samples of sugars and sweeteners at different concentrations

Here, four different disaccharides were studied: sucrose, trehalose, maltose and lactose. Each disaccharide was prepared at eight increasing concentrations and deposited in triplicate in a standard 96-well microplate. Two microplates were used: a first assay was performed with sucrose and trehalose, and the second with lactose and maltose. For each microplate, spectra obtained for the 48 samples were acquired automatically and sequentially using the MultiWell acquisition module of the LabSpec 6 software.

Let's have a look at the first assay dealing with sucrose and trehalose. The obtained spectra were analyzed using a multivariate analysis based on a CLS method as described above. Reference spectra (Figure 4 B) with sucrose in red, trehalose in green and water in blue were acquired and confirmed by the Raman KnowItAll® database provided with LabSpec6 software and powered by Wiley Science Solutions. CLS fitting results are represented in Figure 4 A): each spot corresponds to one well, the spot color corresponds to the component distribution. Depending on the sugar concentration, blue color, corresponding to water, is mixed with red, representing sucrose, or with green, representing trehalose. The results of CLS fitting are named scores and are expressed in percentages of reference component contribution. The scores obtained for sucrose in the sucrose samples and the scores obtained for trehalose in the trehalose samples were correlated to the expected concentrations (Figure 4 C) and Figure 4 D) respectively), and range curves were generated. The score values used are mean values of three replicates. The highest concentrations were excluded as signal saturation was reached.

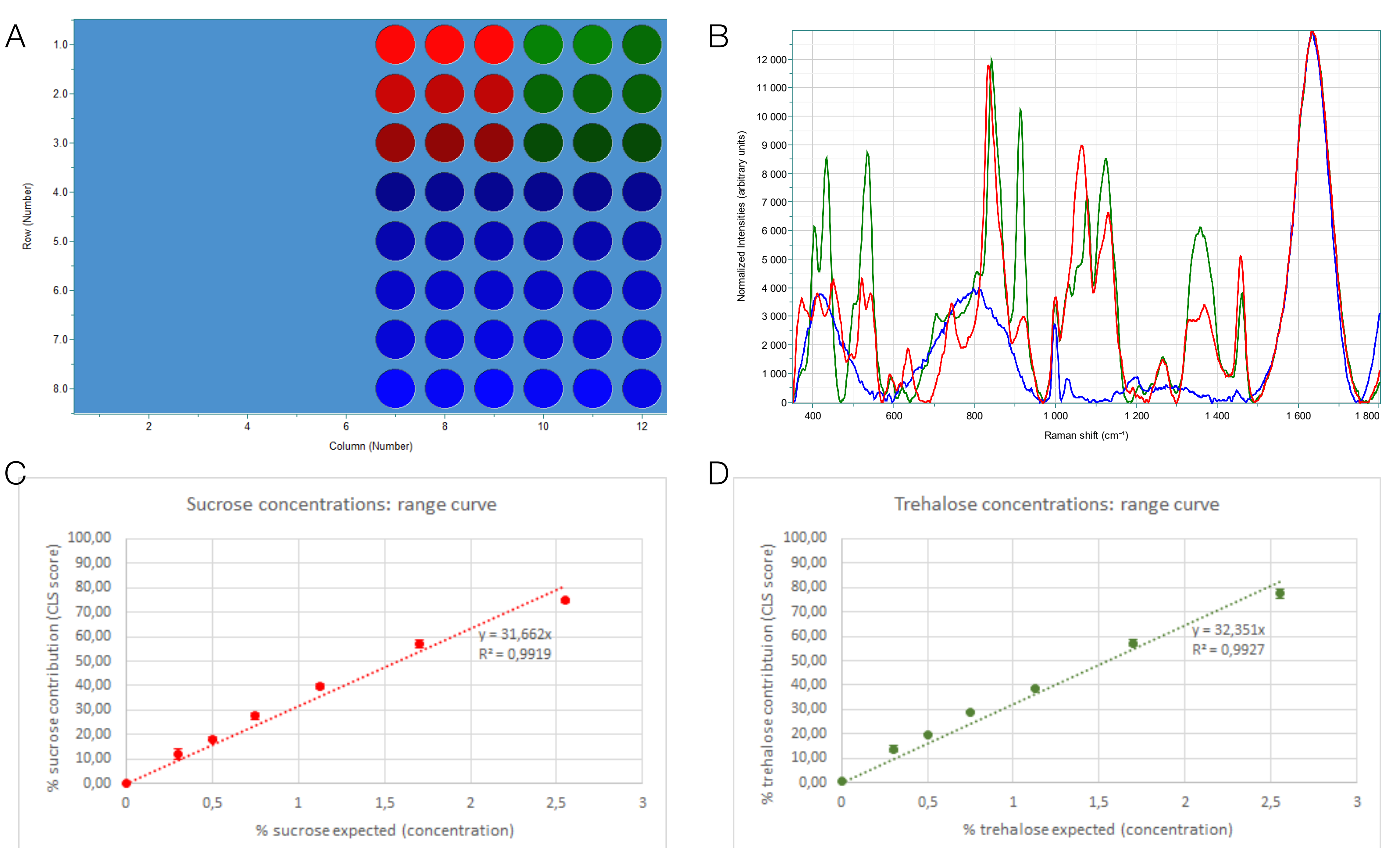

Figure 4: A) MultiWell display results using CLS analysis; B) Reference spectra for CLS analysis; C) Range curve for sucrose and D) Range curve for trehalose. ***Red: sucrose***, ***Green: trehalose***, ***Blue: water***.

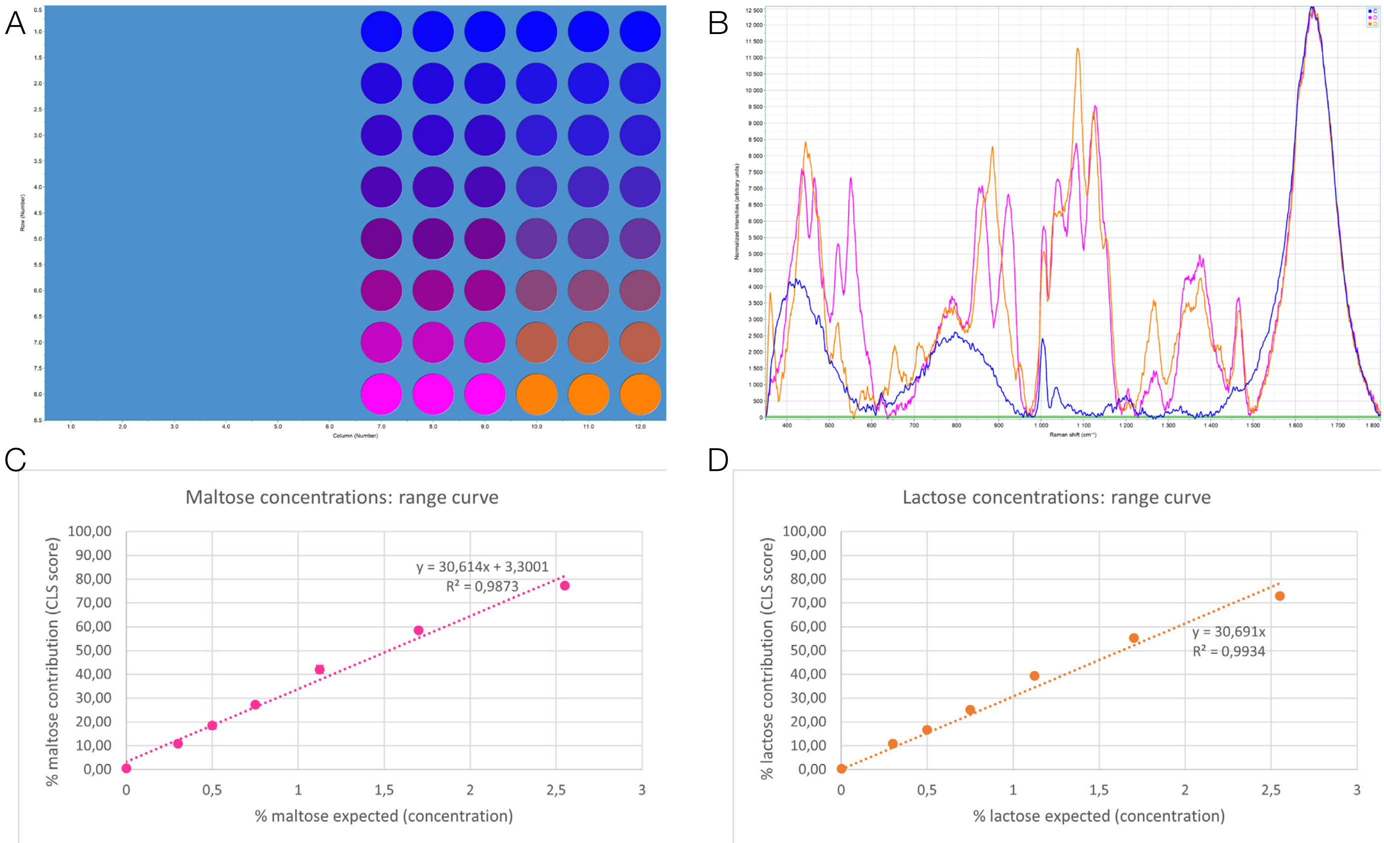

Figure 5: A) MultiWell display results using CLS analysis; B) Reference spectra for CLS analysis; C) Range curve for maltose and D) Range curve for lactose. ***Pink: maltose**, **Orange: lactose**, **Blue: water**.*

Using the CLS analysis, Figure 5 shows results obtained for the second assay, with maltose in pink, lactose in orange and water in blue.

Samples of stevia, a naturally derived sweetener, and synthetically produced aspartame, were prepared at four and five increasing concentrations, respectively. The concentration range for the two sweeteners were deposited in triplicate in a standard 96-well microplate. The obtained spectra were acquired automatically and sequentially using MultiWell acquisition module of the LabSpec 6 software.

The results obtained from the CLS analysis of the samples are shown in Figure 6 and 7 with stevia shown in light blue, aspartame in light green and water in blue. Again, the score values used are mean values of three replicates.

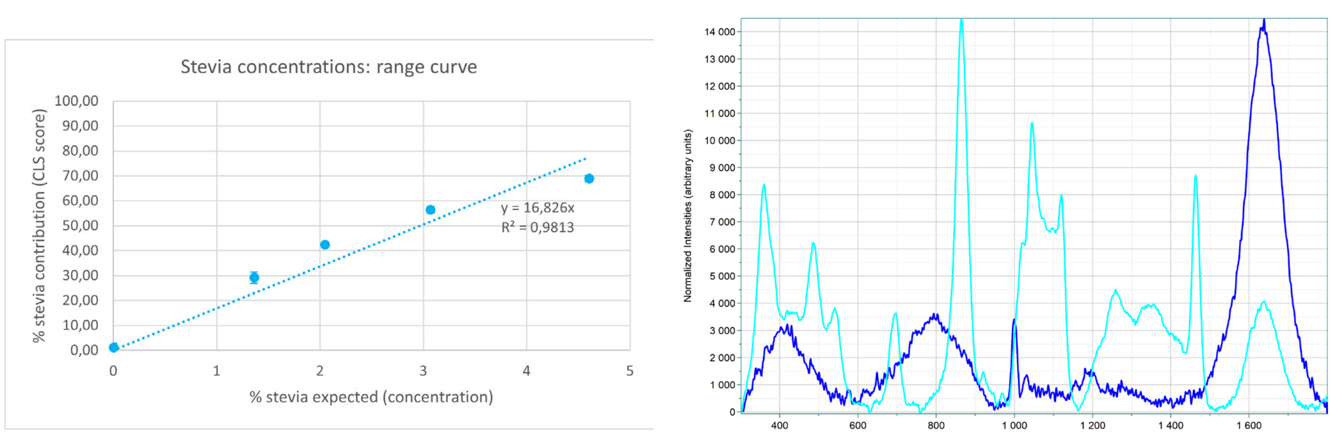

Figure 6: Left: Range curves; Right: Reference spectra for CLS analysis; (Light blue: stevia, Blue: water)

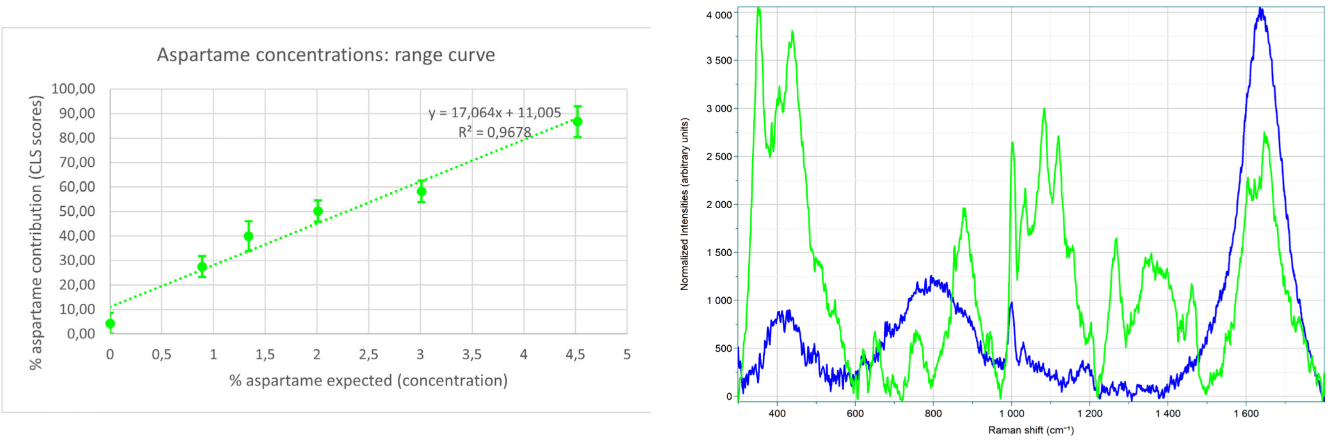

Figure 7: Left: Range curves; Right: Reference spectra for CLS analysis; (Light green: aspartame, Blue: water).

R^2 coefficients obtained for all the calibration curves were between 0.9678 and 0.9934. This means good correlation was obtained for the four sugars and the two sweeteners, demonstrating the efficiency of CLS algorithm.

Applications to common sugary beverages products: citrus soda and orange juice

Two different commercial beverages, a citrus soda and an orange juice were analyzed by Raman spectroscopy. The citrus soda sample was diluted two times and orange juice sample three times in deionized water and each sample was measured in triplicate. The measured data were analyzed using CLS multivariate analysis. An averaged contribution score of a reference component (sucrose) for the two sugary beverages is indicated in Table 1. Using CLS multivariate analysis, the sucrose contents of the beverages were evaluated at around 4% in the citrus soda sample and at around 7% in the orange juice sample, values which are in complete agreement with those indicated by the manufacturing companies.

Table 1: Contribution scores (%) of reference component (sucrose) for two sugary beverages products using CLS multivariate analysis

Citrus soda					
	Replicate			Mean	SD
	1	2	3		
Sucrose at 3.825% wt/v	52.31	53.16	52.48		
Sucrose diluted solution concentration (% wt/v)	2.00	2.03	2.01		
Sucrose stock solution concentration (% wt/v)	4.00	4.07	4.01	4.03	0.03

Orange juice					
	Replicate			Mean	SD
	1	2	3		
Sucrose at 3.825% wt/v	62.24	60.03	60.81		
Sucrose diluted solution concentration (% wt/v)	2.38	2.30	2.33		
Sucrose stock solution concentration (% wt/v)	7.14	6.89	6.98	7.00	0.13

Conclusion

Using Raman spectroscopy, two different types of sugary beverages, a citrus soda and an orange juice, were tested and their sugar concentrations analyzed. This was possible thanks to the powerful MVA Plus multivariate analysis tool. The sugar content of these products must be monitored so it can be communicated to consumers and to label the products in accordance with regulations.

The quality control of these commercial beverages using Raman technique was very simple: samples required almost no preparation and there is no need for a molecular labelling, therefore no consumption of special reagents like there is in liquid chromatography for example, especially solvents.

HORIBA Raman systems enable automated measurements allowing high throughput experiments such as industrial quality control processes. Standard microwell plates are used with the MultiWell module of the LabSpec 6 software to acquire spectra from hundreds of samples sequentially, taking only few minutes for each sample. As an example, with the 96-microwell plates used here, spectra acquisition of almost one hundred samples is easy using a low sample volume of only 200 µL.

References

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