



Comparison of sensitivity to artificial spectral errors and multivariate LOD in NIR spectroscopy – Determining the performance of miniaturizations on melamine in milk powder

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ABSTRACT

This study compared three commercially available spectrometers – whereas two of them were miniaturized – in terms of prediction ability of melamine in milk powder (infant formula). Therefore all spectra were split into calibration- and validation-set using Kennard Stone and Duplex algorithm in comparison. For each instrument the three best performing PLSR models were constructed using SNV and Savitzky Golay derivatives. The best RMSEP values were 0.28 g/100 g, 0.33 g/100 g and 0.27 g/100 g for the NIRFlex N-500, the microPHAZIR and the microNIR2200 respectively. Furthermore the multivariate LOD interval [LOD_{min} , LOD_{max}] was calculated for all the PLSR models unveiling significant differences among the spectrometers showing values of 0.20 g/100 g – 0.27g/100 g, 0.28 g/100 g – 0.54g/100 g and 0.44 g/100 g – 1.01g/100 g for the NIRFlex N-500, the microPHAZIR and the microNIR2200 respectively. To assess the robustness of all models, artificial introduction of white noise, baseline shift, multiplicative effect, spectral shrink and stretch, stray light and spectral shift were applied. Monitoring the RMSEP as function of the perturbation gave indication of robustness of the models and helped to compare the performances of the spectrometers.

Not taking the additional information from the LOD calculations into account one could falsely assume that all the spectrometers perform equally well which is not the case when the multivariate evaluation and robustness data were considered.

1. Introduction

The demand for reliable quality control of dairy products has increased drastically since the uncovering of the illegal addition of melamine in 2008 [1]. The six nitrogen atoms in the chemical structure simulated high nutritional value, which was only possible due to the insensitivity of the Kjeldahl method to the origin of the determined protein – usually being the main source of detected nitrogen content [2]. This food fraud led to kidney failure and nephroliths in hundred thousands of infants – six of them died [3]. Quality control of milk powder has increased dramatically leading to regular safety reports [4], however, since then customers are still aware of this incidence, shown in significant increase of export of i.e. German milk infant formula to China. Especially in China, where this scandal took place, people are still mistrustful against producing industry and prefer imported dairy products [3].

Near Infrared Spectroscopy (NIRS) is a modern and well established technology for a wide variety of applications in food-[5–8],

pharmaceutical-[9,10], petroindustry [11,12] and many more [13]. This technology has the potential of being accessible to the public and contributing dramatically to increasing food safety. NIRS in general has some striking advantages over other vibrational spectroscopic methods such as easy handling, longer path lengths, low operation costs, easy automatization and access to multiple physical as well as chemical parameters [14]. However, the drawbacks are low sensitivity and selectivity combined with complex spectra which require some effort to be interpreted meaningfully. Establishment of reliable calibration usually requires multivariate regression methods such as Partial Least Squares Regression (PLSR), Support Vector Machine Regression (SVR) or Artificial Neural Networks (ANN) to name just a few [15]. There is a huge variety of linear and nonlinear regression methods available that can be studied in detail in the specialized literature [15–17].

Recent technical innovations led to the development of a widely spread field of available spectrometers. The main differences of interest for the end-consumer are price, size, difficulty of handling and reliability of the

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results. Establishing portable NIR spectroscopy might lead to increasing interest of individuals in personalized quality control if they become cheap and easily available. Vibrational spectroscopic methods such as Raman spectroscopy are also capable of identifying food adulterations [18,19], however the present study focuses on NIRS due to it's the recent technical progress in miniaturization and the absence of any lasers which might be harmful for eyesight in private usage.

The application of NIR to melamine adulterated milk powder was investigated by a number of researchers [20–23]. However there is only a very limited amount of quantitative studies regarding portable spectrometers of groceries available [5,24]. Whereas none of them evaluates the usage of those spectrometers critically in regard of the limit of detection/quantification and the robustness of the constructed calibrations models. During this study the performances of different spectrometers were compared in terms of prediction ability of melamine in milk powder (infant formulas). For this purpose two intelligent splitting algorithms (Kennard Stone [25] and Duplex [26]) were applied to compare the influence of differently spread variance from the spectral dataset. The performance of the constructed regression models was additionally evaluated by calculating the multivariate limit of detection. Furthermore robustness of those constructed calibrations regarding parameters such as random noise, baseline offset, multiplicative effect, stray light, wavelength shift and wavelength stretch/shrink, as proposed by Roussel et al., were evaluated by introducing them artificially into the validation set to assess robustness regarding perturbations of the models during a calibration process [27].

The objective of the presented study was to evaluate the influence of the accessible spectral region from each spectrometer, resolution and sample splitting of the data set on the prediction ability of melamine. The constructed models were stressed and the prediction error observed. Furthermore the multivariate LOD for all used spectrometers was calculated.

2. Materials and methods

2.1. Samples

For this study milk powders (infant formula) were purchased from local supermarkets from three different manufacturers. To widen the variety in the sample set a ternary mixture design was applied which resulted in ten individual samples (Fig. 1). Those samples were adulterated with melamine ($\geq 99\%$ Alfa Aesar, ThermoFisher (Kandel) GmbH, Karlsruhe Germany) in a concentration range between 0.0% and 5.5% (11 levels from 0.0% to 5.5%). The total number of samples

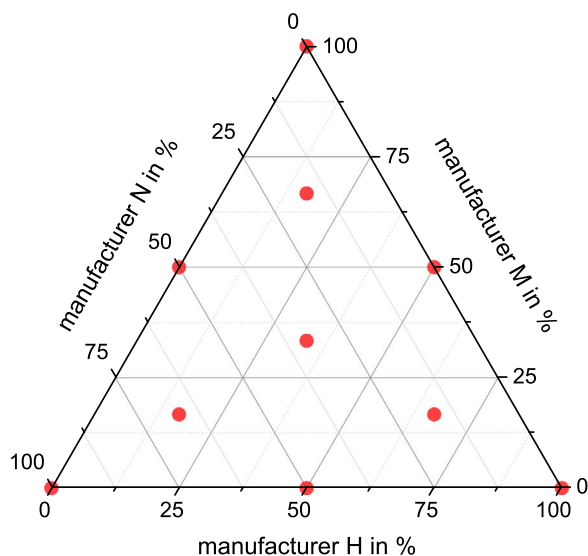


Fig. 1. Ternary mixture design of the produced samples.

was therefore $n=110$ ($=10 \times 11$). The adulteration was done by weighting – leading to an uncertainty of the reference value of 0.01 mg. The production of the samples was performed randomly to avoid systematic errors. To assure the homogeneity of the produced samples the unadulterated and adulterated mixtures were shaken in a 50 ml falcon tube together with an iron sphere for ten minutes. Furthermore, rotation of the cuvette during the NIR measurement was used to reduce small possible differences of the scanned cuvette. Taking samples from different positions/depths of one tube did not show significant differences in the recorded spectra whereas homogeneity was assumed.

2.2. HPLC analysis

The purchased samples were shown to be free of melamine by HPLC analysis. Separation was carried out using a Jupiter 5 u C4 300A column with 150×2.0 mm on a Nexera-I LC2040C-3D system (Shimadzu Group, Nishinokyo Kuwabara-cho, Japan). Sample preparation was performed as described by Gopalakrishnan Venkatasami et al. [28]. As eluent a 95:05 composition from citric buffer (10 mM trisodiumcitrate dehydrate ($\geq 99.0\%$, Fluka Chemika), 10 mM citric acid ($\geq 99.0\%$, Merck Schuchardt OHG) and 10 mM 1-octanesulfuric acid sodium salt ($\geq 99.0\%$, Fluka Chemika)) and acetonitrile ($\geq 99.98\%$, Carl Roth) was used with a flow rate of 0.6 ml/min and 40°C column temperature. 240 nm was used as detection wavelength as described in literature [28,29]. Melamine eluted at 1.8 min. Chromatograms can be seen in [supplementary information \(S1\)](#) for all samples. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were calculated as defined in DIN32645: 2008-11 and given by Eqs. (1) and (2) from the calibration curve (0.5–5 ppm S2). The LOD where $s_{y,x}$ is the standard deviation of y around the regression line, b being the slope of the line, t being the student factor for 95% one sided, N being the number of calibration points, n being the number of repetitions per measurement, \bar{x} being the squared mean of the x-values and $\sum_{i=1}^n (x_i - \bar{x})^2$ was calculated [30]. The LOQ is calculated iteratively due to the presence of the LOQ in the formula itself. The value k is introduced as 3, as suggested by DIN32645: 2008-11, and using t two sided for 95%. The LOQ convergences quickly but was used at the point where the percental change from one iteration to the next was below 0.0000001% [31].

$$LOD = \frac{s_{y,x}}{b} * t_{1-\alpha,v} * \sqrt{\frac{1}{N} + \frac{1}{n} + \frac{\bar{x}^2}{\sum_{i=1}^n (x_i - \bar{x})^2}} \quad (1)$$

$$LOQ = k * \frac{s_{y,x}}{b} * t_{1-\alpha,v} * \sqrt{\frac{1}{N} + \frac{1}{n} + \frac{(LOQ - \bar{x})^2}{\sum_{i=1}^n (x_i - \bar{x})^2}} \quad (2)$$

2.3. Spectrometers

Three different types of spectrometers were used during this study. The benchtop device NIRFlex N-500 (Buchi AG, Flawil, Switzerland) was used with the solids measurement cell with the vial add-on. This spectrometer has a spectral range of $10.000\text{--}4000\text{ cm}^{-1}$ with a wavenumber accuracy of $\pm 2\text{ cm}^{-1}$ and a relative reproducibility of 0.2 cm^{-1} . The external reference was taken at the beginning of the measurements of each day. Spectra recorded with this device had 1501 variables and a resolution of 8 cm^{-1} but a data point interval of 4 cm^{-1} . 32 scans were co added to one average spectrum, after this procedure the sample vial was shaken and scanned again. This procedure was repeated 9 times for each individual mixture. All measurements were averaged resulting in one representative spectrum for every melamine adulteration level per sample.

The handheld spectrometer microPHAZIR GP 4.0 (ThermoFisher Scientific Inc., Waltham, MA, USA) has a spectral range of $6267\text{--}4173\text{ cm}^{-1}$ which is covered by 100 variables leading to an average

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