Title

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Development and validation of an at-line fast and non-destructive Raman spectroscopic method for the quantification of multiple components in liquid detergent compositions

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Abstract

Implementation of process analytical technology (PAT) tools in the manufacturing process of liquid detergent compositions should allow fast and non-destructive evaluation of the product quality. The aim of this study was to develop and validate a rapid method for quantifying the chemical compounds of five washing liquid precursors. Raman spectroscopy was applied in combination with a two-step multivariate modeling procedure. In first instance, a SIMCA (Soft Independent Modeling of Class Analogy) model was developed and validated, allowing the distinction between the different laundry detergents. Once the product was correctly identified, it was aimed at predicting the concentration of its individual components using partial least squares (PLS) models. Raman spectra were collected at-line with a total acquisition time of 20 seconds, using a non-contact fiber-optic probe.

The SIMCA model was perfectly capable of differentiating between the classes of the laundry liquid precursors. Per detergent, the concentration of at least three main ingredients could be predicted with a recovery between 98 % and 102 % and a standard deviation below 2.5 %. Accuracy profiles based on the analysis results of validation samples were then calculated to prove the reliability of the developed regression models. β-expectation tolerance intervals were calculated for each model and for each validated concentration level. The acceptance limits were set at 5 % relative bias, indicating that at least 95 % of future measurements should not deviate more than 5 % from the true value. Furthermore, based on the data of the accuracy profiles, the measurement uncertainty was determined. The developed Raman spectroscopic method demonstrated to be able to rapidly and adequately determine the concentration of the components of interest in the liquid detergent compositions at-line.

Keywords

Process analytical technology

Raman spectroscopy

Chemometrics

Method validation

Measurement uncertainty

1. Introduction

The manufacture of liquid detergent compositions requires a careful balance of ingredients and process steps. Their development and production is based on simple mixing of a number of detergent components. The ingredients may be selected from surfactants, builders, chelants, polymers, organic and inorganic solvents, dyes, perfumes, preservatives, antibacterial agents, viscosity modifiers, pH adjustment agents, water or a mixture thereof. Dosing of these cleaning agent constituents should be performed accurately, as the incapacity of meeting the preferred specifications could negatively impact the cleaning or care ability of the composition. Further, it could adversely affect factors such as physical stability, odor profile, safety profile or regulatory compliance.

Since the publication of the process analytical technology (PAT) guidance by the American Food and Drug Administration (FDA) in 2004, it is generally accepted that quality should be built into products rather than be tested afterwards, preventing the process from being an un-comprehended black box system [1],[2]. Thus, advanced manufacturing practices are being implemented in the pharmaceutical, chemical, biotechnological and food industry, enhancing process efficiency and guaranteeing product quality [3–9]. The consumer goods industry, on the contrary, has been slow at adapting this PAT approach. Nowadays, most consumer goods companies still rely on univariate statistical process monitoring methods (based on univariate sensors) to ensure their product quality. These traditional compliance approaches via product checks by employees have limitations with respect to the number of people required and the accuracy of the checks whilst often missing underlying patterns in process data.

Introducing PAT systems in the manufacturing process of liquid detergent compositions would help to achieve assured high levels of quality and productivity. PAT tools should allow

real-time measurement of key quality parameters in intermediate raw materials and the finished product.

The aim of this study was to develop and validate a fast and non-destructive analytical method for the determination and quantification of the chemical composition of five washing liquid precursors. These intermediates of the liquid detergent production process are mixtures of liquid detergents based on simple blending of cleaning ingredients, to which perfume, dyes and enzymes are added later to create the final washing liquid product. During manufacturing of these liquid compositions, several chemical reactions take place between the combined constituents, so the term mixture is not employed in its purely chemical definition. Whenever the authors refer to a mixture, the liquid composition formed by assembling detergent ingredients is meant, not implying a lack of chemical interaction during production.

A method based on the combination of Raman spectroscopy with multivariate modeling was developed to predict the composition of the complex liquids non-destructively and within a few seconds. Raman spectroscopy is a molecular vibrational spectroscopic technique allowing rapid and non-destructive measurements without sample preparation. Its ability to record spectra directly through transparent glass or plastic packaging and the possibility to quantify compounds in aqueous formulations makes Raman spectroscopy the preferred analytical technique. The list of Raman applications in the pharmaceutical industry seems endless and the use in other industries (e.g. food, forensics, plastic sorting and recycling…) has been growing extensively in the past few years; but again, the consumer goods field is behind on this [3],[4],[10]–[18].

For the multivariate modeling, a two-step approach, consisting of a classification and a quantification phase, was used. During the initial categorization step, a distinction is made between the five types of laundry liquid precursors implemented based on their Raman

spectra, thus identifying the sample in front of the probe. Next, the composition of the complex liquids is checked during the quantification step. More concrete, partial least squares (PLS) models regressing Raman spectra versus the chemical composition of the laundry liquids are fitted for each detergent, allowing to predict the concentration of the washing products main ingredients.

To assure that every future measurement that will be performed in routine will be close enough to the unknown true value of the sample, validation of the quantification method is executed by calculating accuracy profiles. This validation procedure, introduced by the Société Française des Sciences et Techniques Pharmaceutiques (SFSTP) [19],[20] is widely accepted in the pharmaceutical field as can be derived from the numerous applications in literature [10],[21]–[28]. To our best knowledge, no applications are published from the consumer goods industry, making this a cutting-edge approach in the business.

By calculating accuracy profiles, a reliable representation of the methods performance is created, based on β-expectation tolerance intervals. Within day, between day and operator variability are taken into account to estimate the total error of the procedure, influenced by both bias and standard deviation. The objective is to assess the models predictive power, thus minimizing the risk to accept an inaccurate quantification method or reject a capable one.

2. Materials and methods

2.1 Materials

The examined laundry liquid precursors are mixtures of detergent ingredients consisting of 10 to 15 components that are blended together into a homogeneous fluid. The five precursors under investigation differ in terms of compounds present and quantity of these composites. As the proprietary confidential formulas cannot be

concealed, the laundry detergents are simply numbered 1 to 5 and their components of interest are referred to as ingredient A to E in this publication. The target concentrations of these key compounds in the different laundry liquid precursors are listed in table 1.

2.2 Sample preparation

Calibration standards were prepared per laundry liquid precursor according to a central composite circumscribed (CCC) experimental design created in MODDE (Umetrics, Sweden). Since it was aimed at predicting the concentration of a few main ingredients, these key components were introduced as quantitative factors in the design wizard. A range of ± 5 % around the target was set to create the lower and upper concentration level. For detergents 1, 4 and 5, this resulted in the development of a calibration set consisting of 29 lab-made samples. The concentration levels of each ingredient vary on 5 levels within this set, spanning a range between 88 and 102 % of their targeted quantity. Since detergents 2 and 3 contain only 4 of the key ingredients (Table 1), the CCC design of these liquid detergents resulted in a 27-sample calibration set, in which each relative constituent level varies between 90 and 110 %. To take the variability between lab-made and production samples into account, five plant samples produced on target were added to each calibration set, thus resulting in 32 to 34 samples per calibration set.

Six validation standards were created per laundry detergent, with independently varying concentrations of the key ingredients, all deviating ± 1 %, ± 4 % and ± 10 % from their target value. This set was also enlarged with 3 production samples containing all components at their target concentration.

Finally, per detergent, a verification set consisting of 30 randomly selected production samples from different manufacturing dates was used.

Table 2 provides a summary of the composition of the calibration, validation and verification sets per detergent. All lab-made samples were prepared using blends of raw materials from different batches delivered from the production plant.

2.3 Raman spectroscopy

All measurements were performed using a RPA-HE 785 Raman spectrograph equipped with a fiber-optic superhead probe and an air-cooled CCD detector (Horiba, Japan). The spectral data were collected with the accompanying LabSpec spectroscopy software (Horiba, Japan). The laser wavelength during the experiments was the 785 nm line from a diode laser. An exposure time of 1 second with 20 accumulations was selected, leading to a total acquisition time of 20 seconds. PLS_Toolbox version 7.9.3 (Eigenvector Research, Inc., USA) running on MATLAB R2014b (The MathWorks, Inc., USA) was used to analyze the spectra and to develop the classification and calibration models.

2.4 Experimental set-up

The Raman superhead was placed inside a metal case facing a sample holder (Fig. 1A, Fig. 1B). Fixation of the probe in the box ensured robust sample-to-probe presentation. As it was aimed at analyzing the samples at-line, the Raman equipment was installed in close proximity to the process stream. A branch of the production line was lead to the lab, where a plastic cup could be filled with the laundry liquid under production and placed directly inside the metal box (Fig. 1C). The disposable plastic containers fit perfectly inside the sample holder and the cover of the box was closed to prevent interference of ambient light during the Raman measurements. Both production and lab-made samples were prepared by simple blending of the washing liquids raw materials in a fixed order. The lab-made samples were poured into identical plastic cups as the ones on the production site.

2.5 Development and evaluation of the classification model

A soft independent modeling of class analogy (SIMCA) classification model was developed to distinguish between the five types of laundry detergents. The election of this class-modeling method is based on two main advantages over pure pattern recognition techniques such as PLS-DA (Partial Least Squares – Discriminant Analysis). In first instance, SIMCA allows for adding supplementary classes without requiring recalculation of the already existing class models. This is very beneficial since new or improved formulas could easily be implemented without disturbing the current models. Secondly, it is possible to recover samples which are not presented in any of the examined categories. This implies that new samples are not necessarily contributed to a pre-defined class, but might also be categorized as belonging to none of the modeled classes. In this way, irregularities may be traced.

Aiming at covering a broad range of variation sources, all spectral data collected from the calibration, validation and verification sets for the PLS quantification models were utilized for this model development. Using this approach, variations in spectra caused by different concentration levels, raw material batches and production methods (lab-made versus industrial fabrication), as well as day-to-day deviations in instrumental response are taken into account. A new set of 15 production samples was then introduced to test the developed classification model. PLS_Toolbox for MATLAB (Eigenvector Research, Inc., USA) was employed to create the model and evaluate its performance.

The spectral range of 680−3000 cm^{-1} was selected, eliminating the spectral noise at the beginning and end of the spectrum. A standard normal variate (SNV) correction was performed to correct for baseline shifts. The data were mean centered and principal components analysis (PCA) models were fitted using the spectra of each detergent, resulting in five local decomposition models. Evaluation of the classification models performance was executed by plotting the class membership predictions of the test set.

The reduced T^2 and Q values were inspected for each local model, as were the confusion matrix and confusion table of the SIMCA model. Hotelling's T^2 and Q residuals are summary statistics that indicate how well a model is describing a given sample and why that sample has its observed score. Hotelling's T^2 or simplified T^2, is a measure of the variation in each sample within the model. It is calculated as the sum of the normalized squared scores and indicates how variables deviate from the center of the model. Q residuals are a lack-of-fit statistic computed as the sum of squares of each row of the residual matrix that shows how well each sample conforms to the model. The reduced T^2 en Q values are obtained by dividing T^2 and Q by their respective 95 % confidence limit line, which is a normalization that simplifies data interpretation, as a value of 1 always corresponds with the predefined confidence limit. The confusion matrix creates a table showing the true positive, false positive, true negative and false negative rates as a matrix for each class modeled. The confusion table shows the number of samples predicted to belong to the '*i*-th' class, which actually belong to the '*j*-th' class of the classification model.

The selected SIMCA model was then evaluated with a new set of production samples. This independent validation set consisting of 15 randomly selected production samples from different manufacturing dates for each laundry liquid was introduced and the models ability to allocate the detergents to the correct class was inspected. New samples were considered 'in-class' when they were inside the T^2 and Q confidence limit combined. This classification rule first takes the reduced Q and T^2 as explained above and combines the two statistics using the formula (Eq. 1):

$$\text{sqrt}(Q\text{^}2+T^2\text{^}2) \qquad \text{(Eq. 1)}$$

as the distance measure. Only samples inside the sqrt(2) limit are appointed to the class under investigation.

2.6 Development of the PLS quantification models

Once the laundry detergent is identified correctly, the concentration of its individual components is to be predicted using partial least squares (PLS) regression models. Per detergent, 4 to 5 ingredients with a target level varying between 1.8 and 20 % were aimed to be quantified (Table 1). As we aspired adequate determination of the concentration of each individual compound in distinctive matrices, separate regression models were constructed per ingredient per detergent (PLS1). This resulted in a total number of 23 PLS models to be developed. Three Raman spectra were collected per calibration standard. Two different operators performed sample measurements in random order on three different days. The spectral data were regressed against the amount of the component of interest present in the samples. These reference concentration values were calculated gravimetrically from the amount of raw materials weighted during sample preparation, taking the purity of the raw materials into account. First, the calibration, validation and verification sets were investigated individually by fitting PCA models to the spectral data. During this qualitative analysis, abnormalities and outliers could be detected before starting model regression.

For the development of the PLS regression models, several spectral ranges were selected, numerous spectral filters were applied and different numbers of latent variables were chosen for comparison (Table 3).

Determination of the spectral ranges was performed using the VIP (Variable Importance in Projection) plot. This graph gives an estimate of the importance of each variable in the projection used in a PLS model, by summarizing the weights and regression coefficients and taking the explained Y into account. A variable with a VIP score close to or greater than 1 can be contemplated as important in a given model, while variables with VIP scores significantly less than 1 are less important. The latter are considered good

candidates for exclusion from the model, which facilitates the spectral range selection. A variety of ranges going from 120 to 750 variables was thus investigated.

An assortment of spectral filters and their mutual combinations was applied to all data, followed by mean centering.

3 to 10 latent variables were selected based on the inspection of the predictive properties, expressed by the RMSEP values, in function of the order. The RMSEP (Root Mean Square Error of Prediction), is defined by (Eq. 2):

$$\text{RMSEP} = \text{sqrt}\left(\left(\sum_{i=1}^{n}\left(y_{pred,i} - y_{obs,i}\right)^2\right) / n\right) \qquad \text{(Eq. 2)}$$

where $y_{obs,i}$ is the actual value of y for object i, $y_{pred,i}$ is the y-value for object i predicted by the model under investigation and n is the number of objects for which $y_{pred,i}$ is obtained by prediction. In this case, the RMSEP is calculated from the results of the validation and verification samples prediction. The optimal number of latent variables is typically the number at which the addition of another latent variable does not greatly improve the performance of the model (i.e. decrease the RMSEP value).

Using the model optimizer of PLS_Toolbox (Eigenvector Research, Inc., USA), hundreds of regression models were examined for each ingredient. Inspecting the models ability to predict the concentration of the validation and verification samples accurately assessed the performance of the calibration models. Next to the investigation of the RMSEP values, the recovery and relative standard deviation were computed for all samples. The recovery was calculated for each object i as (Eq. 3):

$$\text{recovery} = (y_{pred,i} / y_{obs,i}) \text{ X } 100\ \% \qquad \text{(Eq. 3)}$$

and is expressed in percentages. Then the average recovery was determined for the calibration, validation and verification set, as was the standard deviation on these values.

Based on this information, three model candidates were selected that showed the most promising predictive capabilities.

Nevertheless, these results do not guarantee the models reliability for future application at manufacturing scale. As these traditional model statistics are proven insufficient to validate a method [22–28], the final decision about the most qualified model was made based on the information derived from the accuracy profiles calculated in the validation step.

2.7 Validation of the Raman quantification methods and estimation of their uncertainty

In order to accurately estimate the predictive power of the calibration models in routine, the three candidate models for each ingredient of each complex liquid were investigated in closer detail during this validation step. This resulted in the selection of the final models and the guarantee that future measurements will be close enough to the unknown true value of the component in the sample. It was aimed at including all variability sources that the models might meet during future routine use. Therefore, several concentration levels were investigated, different raw material batches were used for sample preparation and both lab-made and industrial samples were measured by different operators, taking between-day and within-day variability into account.

The harmonized method proposed by the SFSTP is based on the calculation of accuracy profiles, which were conducted with E.noval 3.0 (Arlenda, Belgium). A model was found acceptable when it can be assured that the probability that a measurement (x) will fall outside the predefined acceptance limit (λ) is less than or equal to the risk the analyst is willing to take during routine use. This can be expressed by the following equation (Eq. 4):

$$P(|x-\mu_T| < \lambda) \geq \beta \quad \text{(Eq. 4)}$$

where μ_T is the unknown true value of the sample and β represents the proportion of measurements inside the acceptance limits [19].

In this study, the predefined acceptance limits were set at 5 % relative bias and the maximal risk (1-β) to obtain results outside the tolerance interval at 5 %. The set of 9 validation standards per detergent with varying concentrations of the target ingredients was measured repeatedly on three different times during three different days, by two different operators, resulting in a total of 18 Raman spectra per sample. Estimates of bias and precision were acquired from these data. The accuracy profiles were then obtained by computing the confidence interval that allows evaluating the proportion of expected measures inside the acceptance limits.

The standard deviation of the β-expectation tolerance intervals can be used for assessment of the standard uncertainty in the measurements [29]. The uncertainty is defined as a parameter associated with the result of a measurement, which characterizes the dispersion of the values that could reasonably be attributed to the measurand [30],[31]. The International Organization for Standardization (ISO) suggested in guide 21748 to estimate the measurement uncertainty using repeatability, reproducibility and trueness estimates [31]. The experimental data obtained during the accuracy profile development are perfectly fit for this uncertainty evaluation approach [29].

3. Results and discussion

3.1 Development and evaluation of the classification model

Five local PCA models were fitted to all Raman spectra of the laundry detergent precursors. The spectral range of 680–3000 cm^{-1} was selected, excluding the spectral noise at the beginning and end of the spectra (Fig. 2). After SNV preprocessing followed

by mean centering, the Raman spectra of the five types of laundry liquid are divergent enough to allow a perfect class distinction. 5 to 6 principal components were selected per local detergent PCA model, explaining at least 96 % of the variance in the individual datasets.

The confusion table (Table 4) confirms the correct identification of all external validation samples. This table shows the number of samples that are predicted to belong to a certain class with regard to their actual class membership. No misclassifications are noted, nor is any sample described as not belonging to any class.

3.2 Development of the PLS quantification models

After correct identification of the laundry detergent in the sample holder, the concentration of the liquid detergents main ingredients is to be predicted. PLS models developed from the Raman spectra with different spectral ranges, several pre-processing techniques and a varying number of latent variables were created per component of interest within each laundry liquid. The model performance was then evaluated by inspecting their ability to predict the validation and verification samples correctly.

The optimum number of latent variables for each model was selected after inspection of the RMSEP in function of the number of components. As an example, the model statistics plot of model candidate a predicting ingredient B in detergent 3 is presented in fig. 3. In this illustration, five latent variables were selected, as no significant decrease of the RSMEP was obtained with the addition of extra PLS factors. Fig. 3 also shows the RMSEC (Root Mean Square Error of Calibration) and RMSECV (Root Mean Square Error of Cross Validation) values, which are considered less reliable measures of the predictive power as they are not based on the evaluation of an external test set.

Models were qualified as potential candidates if the recovery of their predictions was found between 98 and 102 % for the calibration, validation and verification sets with a

relative standard deviation below 2.5 % (Fig. 4). For 22 out of the 23 PLS models to be developed, three model candidates meeting those success criteria were selected. Only one component (ingredient A in detergent 3) could not be predicted with such recovery (Table 5). For ingredient A in detergent 3, we did not succeed at developing a satisfying calibration model using the data. This is most probably due to the low detection sensitivity of Raman spectroscopy for this compound.

As the conventional chemometric parameters (such as RMSECV and RMSEP) are insufficient to allow the assessment of the methods ability to predict the content of new samples, accuracy profiles based on tolerance intervals were used as a complementary decision tool to evaluate the models predictive performances.

3.3 Validation of the Raman quantification methods and estimation of their uncertainty

For each of the main ingredients per laundry liquid, three PLS models were selected for a performance evaluation based on accuracy profiles. Then, one final model was selected for which the accuracy calculated through the relative β-expectation tolerance intervals falls entirely within the acceptance limits of ± 5 % (Fig. 5).

As an example, fig. 5 displays the accuracy profile calculated with the validation set results for model candidate a predicting ingredient B in detergent 3. This model has the tolerance limits (dashed blue lines) completely included within the acceptance limits (black dotted lines) over the full concentration range. Here it can be concluded that 95 % of future predictions based on this model will be computed with an error not more than 5 % over the validated concentration range.

For some of the ingredients, all model candidates found their accuracy profiles lying within the acceptance limits. In that case, the model with the lowest risk profile was selected. For other ingredients, only one of the model candidates with a sufficiently good

recovery and standard deviation fell inside the 5 % acceptance limits, confirming the need for a trustworthy validation procedure.

Table 5 gives an overview of the recovery and standard deviation of all final models selected after validation. All of these models had accuracy profiles lying within the predefined acceptance limits over the validated concentration range, guaranteeing that each further measurement of unknown samples will be included within the tolerance limits set at the 5 % level.

Using the data collected for the development of the accuracy profiles, the measurement uncertainty was estimated as suggested by Feinberg et al. [29]. Using this approach, several uncertainty parameters were calculated, which are presented in table 6 for model candidate a predicting ingredient B in detergent 3. The uncertainty of the bias, the uncertainty that combines the uncertainty of the bias with the intermediate precision standard deviation, the expanded uncertainty and the relative expanded uncertainty are displayed for each level of the accuracy profile. The expanded uncertainty defines a 95 % probability interval around the mean value in which the unknown true value is found. Over the entire validated range, the uncertainty of the Raman method is not more than 3.30 %; i.e. the unknown true value is located at a maximum of ± 3.30 % around the result with a confidence level of 95 %.

4. Conclusion

A Raman spectroscopic method was developed to control the dosing of cleaning ingredients for the production of liquid detergent compositions.

The SIMCA classification model was perfectly able to distinguish between the five types of laundry detergents implemented, thus identifying the product in front of the probe correctly. PLS models were developed that predict the concentration of the washing liquids main ingredients with a recovery between 98 and 102 % and a standard deviation below 2.5 %.

Validation of these calibration models by means of accuracy profiles ensured that 95 out of 100 future measurements in routine will be within the acceptance limits of 5 %. Moreover, the data used in this validation approach were used to assess the uncertainty of measurements by estimating the uncertainty of bias as well as the expanded uncertainty at each concentration level.

This Raman spectroscopic method succeeds at evaluating the composition of the laundry liquid precursors in a fast and non-destructive manner and is ready for implementation at-line.

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Table 1. Target concentration of all ingredients to be quantified using PLS calibration models.

	ingredient A	ingredient B	ingredient C	ingredient D	ingredient E
detergent 1	3.894 %	9.009 %	6.597 %	4.687 %	4.821 %
detergent 2	11.252 %	not present	6.902 %	4.247 %	3.982 %
detergent 3	1.876 %	14.710 %	9.406 %	4.158 %	not present
detergent 4	14.904 %	14.869 %	10.829 %	14.189 %	5.012 %
detergent 5	19.959 %	5.249 %	14.771 %	17.090 %	6.034 %

Table 2. Composition of the calibration, validation and verification set of each laundry detergent.

	sample set	sample preparation	number of samples	concentration levels of each key ingredient
DETERGENT 1	**calibration set**	lab-made	29	target, target ± 5 %, target ± 12 %
		production	5	target
	validation set	lab-made	6	target ± 1 %, target ± 4 %, target ± 10 %
		production	3	target
	verification set	production	30	target
DETERGENT 2	**calibration set**	lab-made	27	target, target ± 5 %, target ± 10 %
		production	5	target
	validation set	lab-made	6	target ± 1 %, target ± 4 %, target ± 10 %
		production	3	target
	verification set	production	30	target
DETERGENT 3	**calibration set**	lab-made	27	target, target ± 5 %, target ± 10 %
		production	5	target
	validation set	lab-made	6	target ± 1 %, target ± 4 %, target ± 10 %
		production	3	target
	verification set	production	30	target
DETERGENT 4	**calibration set**	lab-made	29	target, target ± 5 %, target ± 12 %
		production	5	target
	validation set	lab-made	6	target ± 1 %, target ± 4 %, target ± 10 %
		production	3	target
	verification set	production	30	target
DETERGENT 5	**calibration set**	lab-made	29	target, target ± 5 %, target ± 12 %
		production	5	target
	validation set	lab-made	6	target ± 1 %, target ± 4 %, target ± 10 %
		production	3	target
	verification set	production	30	target

Table 3. Model specifications investigated during PLS model development for each component of every laundry liquid.

spectral ranges	spectral filters	number of latent variables
680 – 3000 cm^{-1}	EPO filter	3
680 – 1560 + 2750 – 3000 cm^{-1}	GLS weighting	4
680 – 1830 cm^{-1}	baseline	5
680 – 1820 cm^{-1}	detrend	6
680 – 1720 cm^{-1}	extended scatter correction	7
680 – 1560 cm^{-1}	first derivative	8
730 – 1650 cm^{-1}	second derivative	9
730 – 1160 cm^{-1}	SNV	10
880 – 1700 cm^{-1}	MSC	
980 – 1630 cm^{-1}	normalize	

Table 4. Confusion table of the SIMCA classification model. The header row shows the actual class membership, while the column row illustrates the number of samples that are predicted to belong to a certain class.

	detergent 1	detergent 2	detergent 3	detergent 4	detergent 5	no class
predicted as detergent 1	15	0	0	0	0	0
predicted as detergent 2	0	15	0	0	0	0
predicted as detergent 3	0	0	15	0	0	0
predicted as detergent 4	0	0	0	15	0	0
predicted as detergent 5	0	0	0	0	15	0
predicted as no class	0	0	0	0	0	0

Table 5. Recovery and standard deviation (stdev) of the predictions of the calibration (cal), validation (val) and verification (ver) set of all final models selected.

		INGREDIENT A		INGREDIENT B		INGREDIENT C		INGREDIENT D		INGREDIENT E	
		recovery	stdev	recovery	stdev	recovery	stdev	recovery	stdev	recovery	stdev
DETERGENT 1	cal set	100.02 %	1.24 %	100.01 %	1.04 %	100.02 %	1.36 %	100.00 %	0.66 %	100.00 %	0.37 %
	val set	99.72 %	2.44 %	99.74 %	0.95 %	99.69 %	2.34 %	99.94 %	2.39 %	100.65 %	1.80 %
	ver set	99.68 %	1.68 %	100.44 %	0.67 %	100.74 %	2.23 %	100.09 %	2.00 %	99.88 %	0.92 %
DETERGENT 2	cal set	100.00 %	0.45 %	Not present		100.01 %	1.05 %	100.02 %	1.54 %	100.00 %	0.63 %
	val set	99.49 %	1.17 %			100.75 %	2.00 %	100.32 %	2.00 %	99.80 %	1.79 %
	ver set	100.43 %	0.59 %			99.92 %	1.59 %	100.02 %	2.25 %	98.75 %	0.77 %
DETERGENT 3	cal set	No adequate model found		100.02 %	1.25 %	100.04 %	1.94 %	100.00 %	0.87 %	Not present	
	val set			99.65 %	1.48 %	99.80 %	1.45 %	99.85 %	1.90 %		
	ver set			100.91 %	1.42 %	101.29 %	1.55 %	100.35 %	1.56 %		
DETERGENT 4	cal set	100.00 %	0.55 %	100.01 %	1.08 %	100.00 %	0.55 %	100.00 %	0.41 %	100.04 %	2.01 %
	val set	100.40 %	1.56 %	99.56 %	1.21 %	99.99 %	1.42 %	100.90 %	1.50 %	99.34 %	2.39 %
	ver set	100.14 %	1.64 %	100.16 %	1.05 %	100.05 %	1.26 %	100.36 %	1.08 %	101.25 %	1.61 %
DETERGENT 5	cal set	100.01 %	0.98 %	100.01 %	0.83 %	100.01 %	1.11 %	100.00 %	0.34 %	100.01 %	0.90 %
	val set	100.23 %	0.99 %	100.28 %	1.74 %	100.02 %	0.92 %	100.22 %	0.57 %	99.07 %	1.39 %
	ver set	99.89 %	0.75 %	98.52 %	1.91 %	99.04 %	1.53 %	100.01 %	1.52 %	100.47 %	1.65 %

Table 6. Estimates of the different uncertainties related to the content of ingredient B in detergent 3 at each concentration level of the accuracy profile using the PLS model candidate a.

concentration level	uncertainty of the bias	uncertainty	expanded uncertainty	relative expanded uncertainty
12.50 %	0.06019 %	0.1766 %	0.3532 %	2.777 %
13.53 %	0.05513 %	0.1695 %	0.3391 %	2.509 %
14.27 %	0.04919 %	0.1533 %	0.3066 %	2.154 %
14.71 %	0.07427 %	0.2426 %	0.4852 %	3.298 %
15.15 %	0.07628 %	0.2297 %	0.4595 %	3.077 %
15.89 %	0.05885 %	0.1709 %	0.3419 %	2.194 %
16.92 %	0.07505 %	0.2218 %	0.4436 %	2.676 %

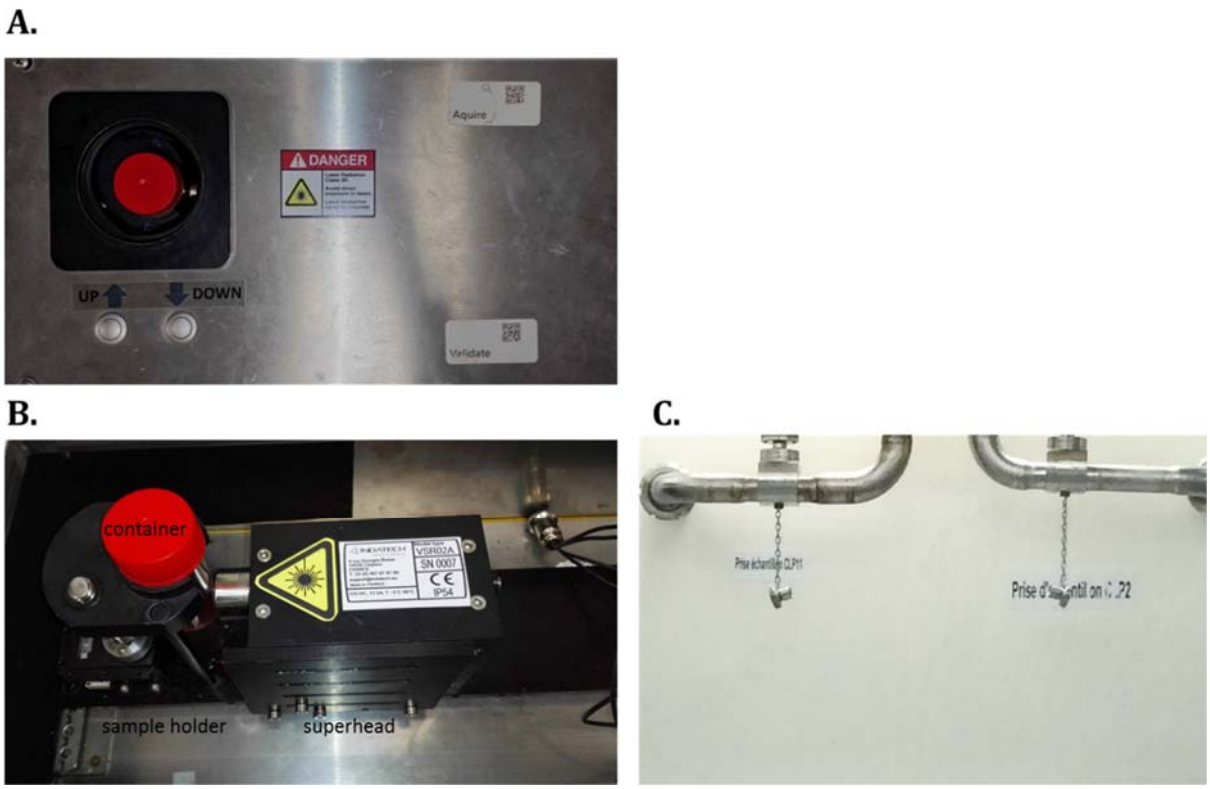


Fig. 1. Experimental set-up: A. Raman superhead enclosed in metal box, with sample in plastic container ready for measurement. B. Content of box: fiber-optic Raman probe facing the sample holder. C. Branch of the production line passing through the lab for sample collection.

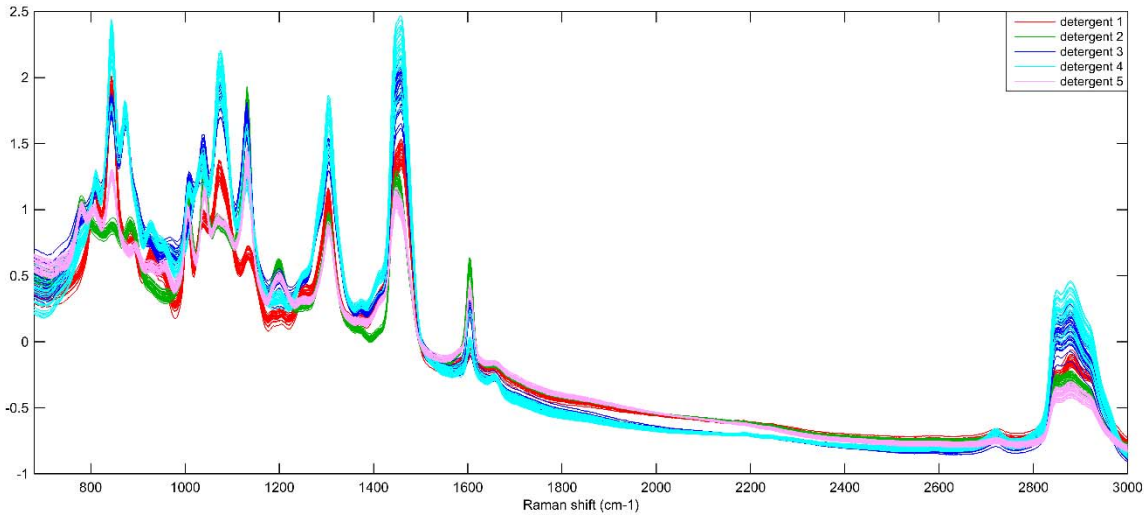


Fig. 2. SNV-corrected spectra of all laundry detergents in the selected spectral range of 680-3000 cm^{-1}, colored according to classes.

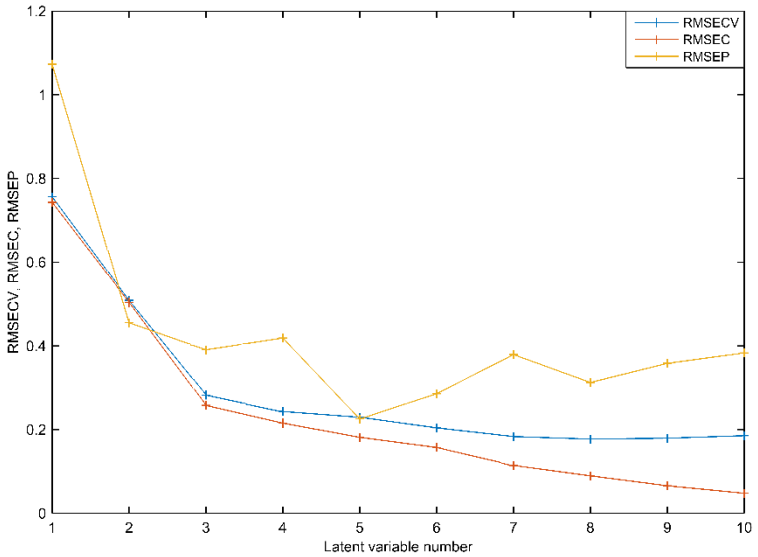
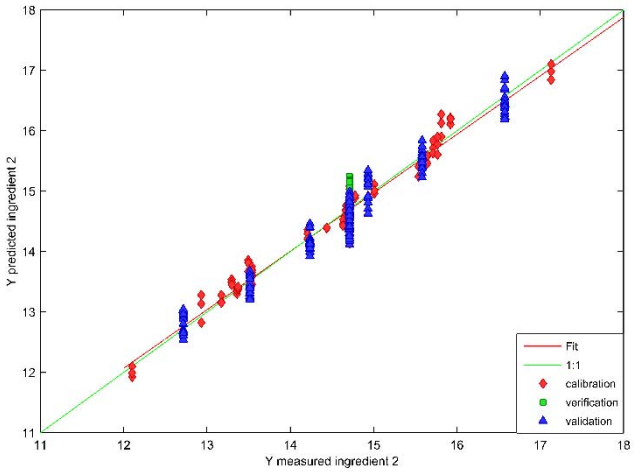


Fig. 3. RMSECV (blue), RMSEC (red) and RMSEP (yellow) values versus the number of PLS factors in model candidate a predicting ingredient B in detergent 3.



	recovery	standard deviation
calibration set	100.02 %	1.25 %
validation set	99.65 %	1.48 %
verification set	100.91 %	1.42 %

Fig. 4. A. Example of an observed versus predicted plot of model candidate a predicting ingredient B in detergent 3. B. Recovery and standard deviation values of the model candidate.

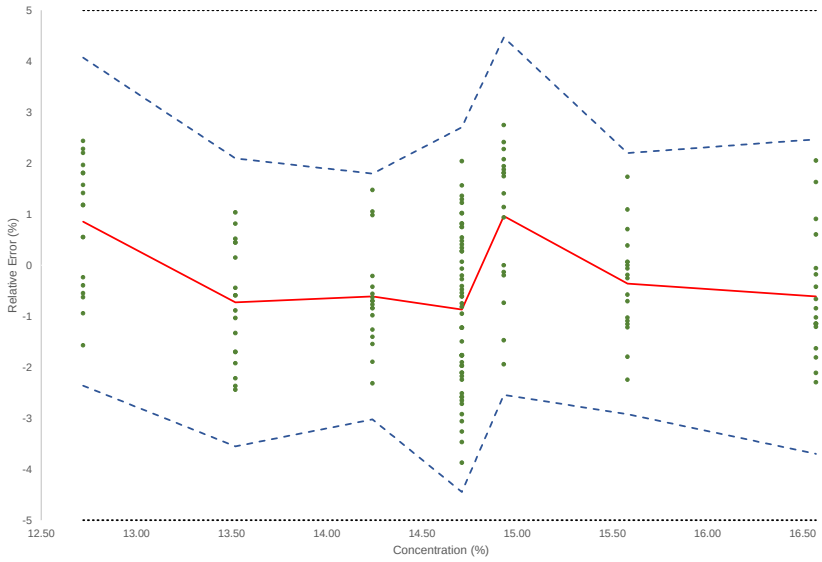


Fig. 5. Accuracy profile of model candidate a predicting ingredient B in detergent 3. The black dotted lines represent the acceptance limits, which were set at 5 % relative bias. The dashed blue lines are the β-expectation tolerance limits at each validated concentration level. The green dots represent the relative error of the back-calculated concentrations and are plotted with respect to their targeted concentration, while the plain red line shows the relative bias.