Qualitative and Quantitative Methods for the Quality Control of Ethanol-Based Hand Sanitizers using a Low-Cost Transmission Near Infrared Spectrometer

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Alcohol-based hand sanitizers are essential for hand hygiene and help prevent the spread of infectious diseases. This work focuses on the development and validation of qualitative and quantitative methods for quality control of ethanol-based disinfectants using a low-cost handheld transmission spectrophotometer and chemometrics. Calibration and validation samples, in liquid and gel forms, were prepared with ethanol concentrations ranging from 40 to 90 % v/v. The qualitative method involved constructing a model based on data-driven soft independent modeling of class analogy, enabling the identification of hand sanitizers with an ethanol concentration between 60 and 80% v/v. A sensitivity of 100 % was achieved for both the training and validation sets. The evaluation of the specificity of the constructed model showed satisfactory results when analyzing samples containing ethanol at lower and higher concentrations, as well as samples containing other alcohols. The quantitative method was based on a partial least squares regression model for the determination of ethanol in hand sanitizers. The validation study, conducted using the accuracy profile strategy, showed that the developed method was specific, linear, fair, precise, and accurate over the ethanol concentration range of 40-90 % v/v. The developed and validated methods were successfully used to analyze various commercial ethanol-based disinfectants.

Keywords: handheld NIR spectrophotometer, total error approach, hand disinfectant, DD-SIMCA, PLS

Introduction

According to the World Health Organization (WHO), an alcohol-based hand rub is defined as an alcohol-containing preparation designed for application to the hands to inactivate microorganisms and/or temporarily suppress their growth [1]. It is an important product for hand hygiene, which prevents the spread of infectious diseases. Alcohol-based sanitizers are usually in liquid or gel form and may contain water, one or more types of alcohol, and other compounds such as humectants, emollients, or gelling agents [1, 2]. Ethanol is the most commonly used alcohol in hand sanitizers and is most effective against microbes and viruses at concentrations of 60-80% v/v [3, 4]. Hand disinfectants have been widely used during the COVID-19 pandemic. Indeed, health authorities have recommended the use of an alcohol-based hand sanitizer as an alternative to prevent Coronavirus transmission when soap and water are not available [2, 5]. With high demand, many products of various origins and of poor quality have appeared on the market, particularly in low- and middle-income countriesseveral irregular factories emerged requiring an efficient control by the police and regulatory agencies to guarantee product quality.

Alcohol content is the main quality criterion of an alcohol-based sanitizer. Ethanol concentration in hand sanitizers is generally determined using an alcoholmeter or a gas chromatography method. However, an alcohol meter is not suitable for analyzing gel products, and gas chromatography analysis is expensive, destructive, and time-consuming [10]. Among alternative techniques, near-infrared (NIR) spectroscopy, when combined with chemometrics, offers the advantages of being nondestructive, fast, requiring little or no sample preparation, and environmentally friendly [11,12]. In addition, NIR spectrometers have been miniaturized with the

emergence of cost-effective portable devices, thereby facilitating their use in laboratories with limited resources [11,13]. In the literature, some methods based on a portable NIR spectrometer combined with chemometrics are described for qualitative analysis [10] or quantitative analysis [6, 14-15] of hand sanitizers, but, to our best knowledge, no published study provides both quantitative and qualitative quality control. In addition, these quantitative methods were not validated in accordance with the guidelines for analytical method validation. Indeed, the performance of the developed methods was evaluated using only the root mean square error of prediction (RMSEP). This is not enough to guarantee that the method is suitable for the intended purpose in routine use [16]. As NIR spectroscopy is an analytical technique, it should be fully validated by evaluating specificity, linearity, precision, trueness, and accuracy.

The aim of the presented work was to develop and validate qualitative and quantitative methods using a low-cost handheld transmission near infrared spectrometer for the quality control of ethanol-based sanitizers in liquid and gel form. The spectrometer used is equipped with a cuvette holder enabling direct spectral acquisition of liquid samples without the need for any laboratory-made accessories. Calibration and validation samples were prepared in two formulations (liquid and gel) with ethanol content ranging from 40 to 90 % v/v. The qualitative method consisted of building a model, based on data-driven soft independent modeling of class analogy (DD-SIMCA), to identify hand sanitizers with an ethanol concentration between 60 and 80% v/v. The specificity of the constructed model was assessed by analyzing samples containing ethanol at lower or higher concentrations, as well as samples containing other alcohols to mimic falsified and substandard disinfectants. The quantitative method was based on the development of a partial least squares (PLS) regression model for quantifying ethanol at concentrations of 40-90 % v/v. The PLS model was validated using the accuracy profile method, which fully complies with the International Council for Harmonisation (ICH) requirements for the validation of analytical methods. Both qualitative and quantitative methods were employed to analyze various ethanol-based hand sanitizers collected in Burkina Faso.

Materials and methods

Chemicals

The ethanol used (purity ≥ 99,9 %) was from Scharlau (Barcelona, Spain). Glycerol 98 % and hydrogen peroxide 3% were kindly provided by the Teaching Hospital Sourô Sanou (Bobo-Dioulasso, Burkina Faso). The blank gel was prepared and provided by the drug development laboratory at Joseph Ki-Zerbo University (Ouagadougou, Burkina Faso). It was composed of carbomer 980 used as a gelling agent at a concentration of 0.5 % in water and triethanolamine used as a neutralizing agent.

Instrumentation

Analyses were performed using a low-cost handheld transmissive near infrared spectrometer, NIR-M-T1, from Innospectra Corp. (Taiwan). The apparatus has a cuvette holder for spectral acquisition of liquid samples in the wavelength range of 900-1700 nm. Each acquired spectrum was an average of 32 scans, with a digital resolution of 228 and a PGA gain of 16. The lamp was operated for 1 hour prior to the analysis to ensure a stable detector temperature. Thereafter, the background was recorded, and the lamp remained activated throughout the analysis. The apparatus was subsequently turned off and allowed to cool to ambient temperature between each validation series. For spectral acquisition, samples were transferred into a Hellma UV quartz cell (light path, 2 mm), which was then placed in the cuvette holder of the spectrometer. For each individual sample, four spectra were measured.

Preparation of calibration and validation samples

Calibration samples were used to build models, while validation samples were used to evaluate the developed models. Calibration and validation samples were prepared according to two formulations adopted worldwide (Table 1). Formulation 1 was a liquid preparation based on the WHO formula, while formulation 2 was a gel preparation [14,17]. Six ethanol concentration levels (40 %, 50 %, 60 %, 70 %, 80 %, and 90 %, v/v) were used for each formulation and for both calibration and validation sets. For formulations 1 and 2, 2 mL of each sample was prepared by mixing the appropriate amounts of ethanol, water, and other additives.

Table 1. Composition of sanitizers according to the formulations 1 and 2.

Components	Form. 1 % v/v	Form. 2 % v/v
Ethanol	80	70
Water	14.4	29.5
Glycerol 98 %	1.4	-
Hydrogen peroxide 3 %	4.2	-
Carbomer 980	-	0.5
Triethanolamine	-	Quantum satis pH 5

To facilitate the preparation of samples for formulation 2, a blank gel composed of water, carbomer (as a gelling agent), and triethanolamine (as a neutralizing agent) was prepared. Then, the appropriate amount of ethanol was mixed with an adequate amount of the blank gel to obtain 2 mL of the sample. For each concentration level and formulation, samples were prepared in triplicate for both calibration and validation sets. Four series (one per day) were

prepared, totaling 144 samples per calibration and validation set (288 samples in total). For each sample, four spectra were acquired. Therefore, a total of 1152 spectra were obtained for both calibration and validation sets.

Chemometric analysis

Spectral preprocessing

Before chemometric analysis, acquired spectra were preprocessed to remove irrelevant information and enhance the signal-to-noise ratio. Various pre-treatments were evaluated using the Savitzky–Golay (SG) algorithm, with or without a derivative, and followed by, or not by, the standard normal variate (SNV) transform. Pre-processed spectra were mean-centered or scaled before multivariate analysis.

Qualitative analysis

Prior to the development of the quantitative model, a one-class classifier (OCC) model, such as DD-SIMCA, for identifying non-conforming sanitizers has been investigated. DD-SIMCA functions as a dual principal component analysis (PCA) /SIMCA [18-20]. It begins by applying PCA to the training dataset of the target class. Subsequently, the PCA outcomes are used to compute a score distance (*hi*) and an orthogonal distance (*qi*) for each sample in the training set. The contribution of the sum of the two distances *hi* and *qi*, whose values follow a chi-square distribution, is used to estimate the full distance (*fi*) of each object. When the full distance of an object is less than or equal to the critical full distance $f_{critical}$ (which is determined using the chi-squared quantile function for a given type I error α and the corresponding number of degrees of freedom), that object is categorized as belonging to the target class [19,20]. The DD-SIMCA results can be represented in a two-dimensional plot using the coordinates $ln\ (1 + h/h_0)$ versus $ln\ (1 + q/q_0)$, with an acceptance area that allows determination whether the samples belong to the target class [13]. The principal metric for evaluating the performance of an OCC is sensitivity, defined as the percentage of samples belonging to the target class that are correctly recognized as such by the model [19].

The target class was composed of samples with ethanol concentrations between 60 and 80 %, v/v. This concentration range was selected because it has been shown that ethanol's viricidal potential occurs within this range [3]. Spectra of calibration samples at the concentration between 60 and 80 % v/v (108 spectra) were used as a training set to build the DD-SIMCA model. The type I error α was set at 0.01. An external validation was carried out using a test set composed of spectra from independent validation samples at concentrations between 60 and 80 % (v/v). Sensitivity values were computed for the training and validation sets. To evaluate the model's ability to reject non-members of the target class, spectra of samples at the other concentration levels (40 %, 50 %, and 90 %) were analyzed using the built model. In addition, samples composed of distilled water, absolute ethanol, methanol, and 70 % (v/v) isopropanol were analyzed. These samples were used to mimic substandard and falsified hand sanitizers and to calculate specificity (the percentage of samples from non-members of the target class that are correctly classified as non-members by the model) [21].

Quantitative analysis

To quantify ethanol in hand sanitizers, spectra of calibration and validation samples from the two formulations were used to build and validate a partial least squares (PLS) regression model. The number of latent variables (LV) was selected based on the root mean square error of prediction (RMSEP) as the quality criterion. The built PLS model was then fully validated using the total error approach, which fully complies with the ICH Q2 guideline requirements [22,23]. The validation parameters were specificity, linearity, precision (repeatability and intermediate precision), trueness, and accuracy profile with the acceptance limits of ±10% and a 5% risk level.

Analysis of commercial hand sanitizers

The developed and validated qualitative and quantitative NIR methods were used to analyze 24 samples of commercial hand sanitizers collected in the town of Bobo-Dioulasso (Burkina Faso). Each sample was directly transferred within the Hellma UV quartz cell without any sample preparation. The cell was then placed in the handheld NIR spectrometer's sample holder for spectral acquisition. For each sample, four spectra were acquired. For the quantitative analysis, a sample was considered compliant when the stated content (ratio of the measured concentration to the nominal concentration stated on the sanitizer label) was between 90 and 110 %. The ethanol content of liquid disinfectants was also determined using an alcoholmeter and compared with the results obtained with the NIR method.

Software

The software used for spectral data acquisition was ISC_NIRScan_GUI (version 3.7.13, Innospectra Corp., Taiwan). Spectral preprocessing, principal component analysis, and PLS modeling were performed using the PLS_Toolbox version 8.9.2 (Eigenvector Research Inc., USA), which was integrated within the MATLAB environment, version R2019a (MathWorks Inc., USA). The DD-SIMCA analysis was performed using version 2.0.4 of the web application (https://www.mda.tools/ddsimca/).

Results and discussion

Preprocessing

The raw spectra of calibration samples of both formulations 1 and 2 are shown in Figure 1a, together with the raw spectra of absolute ethanol and distilled water. To remove irrelevant information and enhance the signal-to-noise ratio, acquired spectra were pre-processed before chemometric analysis. The pre-processing method that yielded the best results

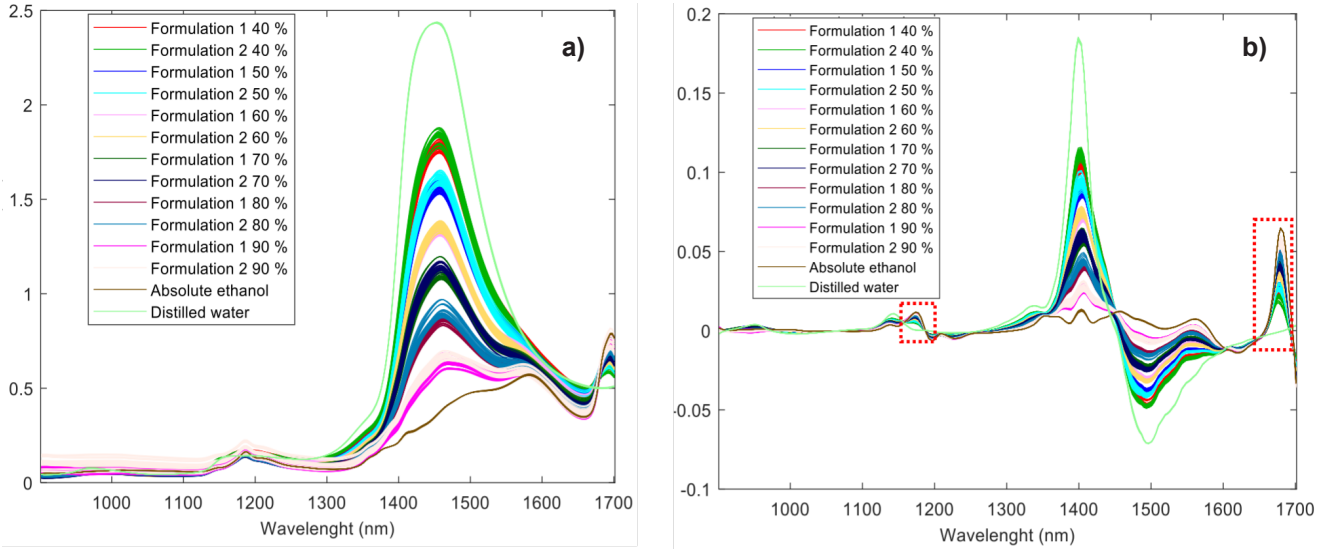

Figure 1. Raw (a) and preprocessed spectra (b). The selected spectral ranges are framed in red.

during our investigation was the Savitzky–Golay first derivative with a second-degree polynomial and a 7-point window (Figure 1b). Water showed strong absorption in the 1300-1600 nm range, masking the absorption of ethanol. The spectral ranges in which the feature of ethanol is highlighted with a low water absorption were found to be 1158–1203 nm, 1667–1689 nm (Figure 1b). These spectral ranges were selected for building the qualitative and quantitative models.

A classification model for identifying hand sanitizers with ethanol content between 60 and 80 % was investigated. A one-class classifier, such as DD-SIMCA, was used. The training set consisted of spectra of calibration samples from both formulations 1 and 2, with ethanol content between 60 and 80 %. The significance level α was set at 0.01, and the model was built using 2 principal components (PC). A sensitivity of 100 % was achieved in the training set (Figure 2a). The external validation using independent samples also showed a sensitivity of 100 % (Figure 2b). The application of the model to alternative classes (samples with ethanol contents of 40 %, 50 %, and 90 %) used to mimic substandard sanitizers also showed satisfactory results (Figure 3). Indeed, all samples with ethanol concentrations of 40 % and 90 % were rejected by the model as non- target samples, indicating a specificity of 100 % (Figure 3a). For the samples at ethanol content at 50%, a specificity of 83 % was achieved. In addition, samples containing 70 % methanol and isopropanol were rejected by the model as non-members of the target class (Figure 3b). The results showed that the built DD-SIMCA model was suitable for identifying hand sanitizers with ethanol content between 60 and 80 % v/v.

Quantitative analysis model

PLS model development

To determine ethanol content in disinfectants, a PLS model was built using preprocessed spectra of calibration and validation samples in the 1158–1203 nm and 1667–1689 nm wavelength ranges. The ethanol concentration range was 40% to 90%. The number of latent variables retained was four (04). The RMSEC, RMSECV, RMSEP, and the prediction coefficient of determination R^2 were 1.80 %, 1.81 %, 1.57 %, and 0.9961, respectively. The low RMSEP and an R^2 value close to 1 suggest good performance of the PLS model. However, this is not sufficient to guarantee that the developed method is adequate for determining ethanol content in sanitizers. Indeed, since NIR spectroscopy is an analytical technique, it should be fully validated by assessing the specificity, linearity, precision, trueness, and accuracy.

Method validation

The validation procedure was performed according to the accuracy profile methodology, which fully complies with the ICH Q2(R1) requirements for the validation of analytical methods [22, 23].

The specificity of the quantitative PLS method was demonstrated during the development of the qualitative model. Indeed, the constructed DD-SIMCA model enabled clear authentication of sanitizers containing ethanol, differentiating them from samples composed of distilled water or other alcohols, such as methanol and isopropanol (Figure 3b). Furthermore, comparing the PLS regression vector with the preprocessed ethanol spectrum shows that the ethanol features align with the regression vector. This observation can be interpreted as confirmation of the specificity of the regression model's specificity (Figure 4).

The results of the evaluation of linearity, precision, trueness, and accuracy are presented in Table 2. Linearity was assessed by computing a linear regression of the predicted concentrations (using the PLS model) against the introduced concentrations across the assay range. The linear regression analysis showed a slope close to unity and a coefficient of determination higher than 0.99. Also, as illustrated

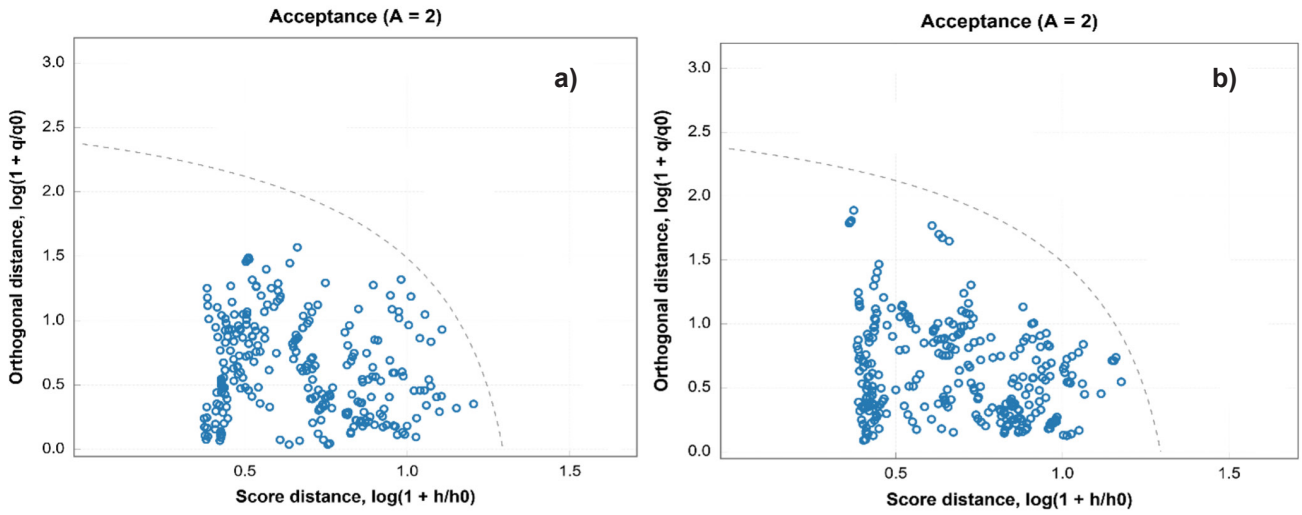

Figure 2. DD-SIMCA acceptance plots of the target class (ethanol content between 60 and 80 %) with 2 PCs (A = 2). (a) Samples of the training set. (b) Samples of the validation set. The dashed line delimits the acceptance area. Samples are deemed to belong to the target class if they fall within the acceptance area.

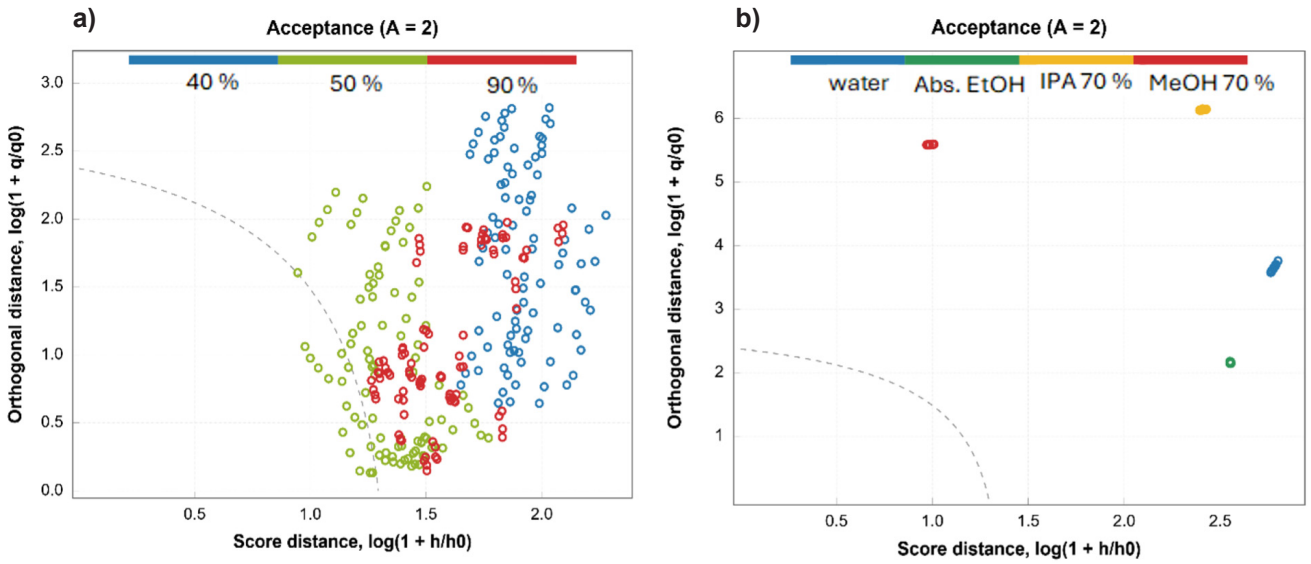

Figure 3. DD-SIMCA acceptance plots of alternative classes. (a) Sanitizer samples with ethanol contents of 40%, 50%, and 90%. (b) Samples of water, absolute ethanol (Abs. EtOH), isopropanol (IPA) at 70 %, and methanol (MeOH) at 70 %. The dashed line delimits the acceptance area. Samples are deemed to belong to the target class if they fall below the acceptance area. Samples are considered non-members of the target class if they fall outside the acceptance zone.

in Table 2, the calculated experimental F value (the ratio of residual variance to squared pure error) was lower than the tabulated critical F value at the 5% significance level, thereby demonstrating the linearity of the developed quantitative method [24]. Relative standard deviations (RSD%) for repeatability and intermediate precision were below 2 %, indicating good precision for the method. The trueness of the method was found satisfactory since the relative biases were below 1 % at each concentration level. The β-expectation tolerance intervals at the 95 % confidence level were within the acceptance limits (±10 %), indicating that the NIR method provided accurate results across the concentration range. The resulting accuracy profile is presented in Figure 5. The results demonstrated the suitability of the developed PLS method for the intended purpose: quantifying ethanol in hand sanitizers.

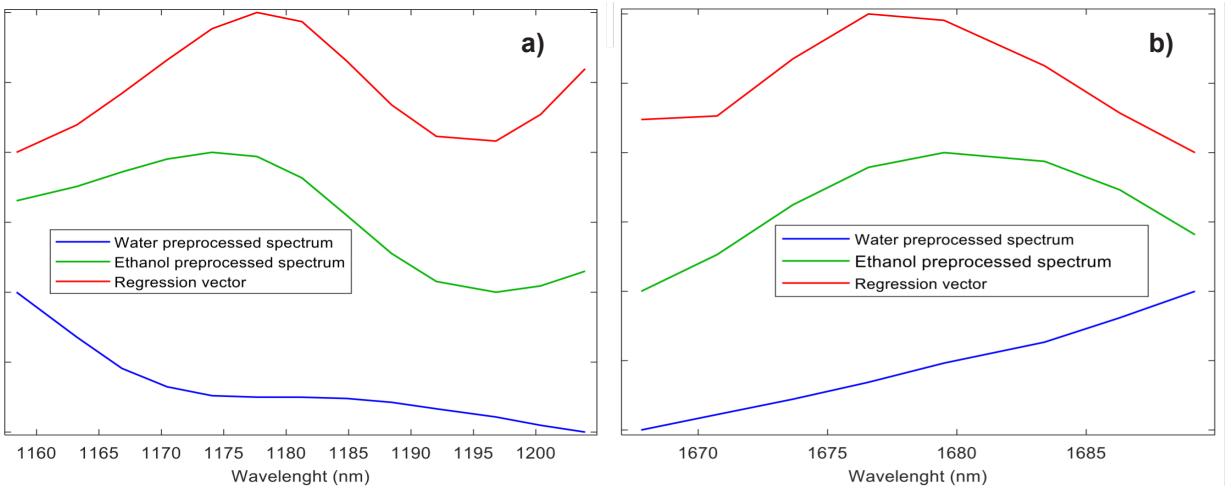

Figure 4. Regression vector presented with the preprocessed spectra of water and ethanol in the spectral range of 1158-1203 nm (a) and 1667-1689 nm (b). The spectra are offset to improve visual discernibility. One can see that the regression vector has a similar profile to the preprocessed spectrum of ethanol, but is very different from that of water. This observation may be considered confirmation of the specificity of the PLS model to ethanol features.

Table 2. Validation results of the developed PLS regression method.

Validation Criteria		
Linearity	Slope	1.0003
	Intercept	0.3881
	R^2	0.9963
	Residual variance	0.7300
	Squared pure error	0.7640
	F_{exp}	0.9555
	Critical F (0.05, 106, 102)	1.3836
Trueness	Concentration level (% v/v)	Relative bias (%)
	40	0.76
	50	0.70
	60	1.20
	70	-0.37
	80	0.53
	90	0.41
Precision	Concentration level (% v/v)	Repeatability (RSD %)/ Intermediate Precision (RSD%)
	40	1.57/1.79
	50	1.61/1.61
	60	0.92/1.14
	70	1.18/1.48
	80	1.29/1.29
	90	1.79/1.79
Accuracy	Concentration level (% v/v)	β-expectation tolerance limits (%)
	40	[-3.36, 4.89]
	50	[-2.88, 4.28]
	60	[-1.53, 3.93
	70	[-3.15, 3.90]
	80	[-2.35, 3.42]
	90	[-3.59, 4.42]

F_{exp} is the ratio of residual variance to squared pure error, critical F (0.05, 106, 102) is the critical value of F with (I – 2) = 106 and (I – L) = 102 degrees of freedom at 95% confidence level, where I is the number of samples used to plot the linearity (108) and L the number of concentration levels (6).

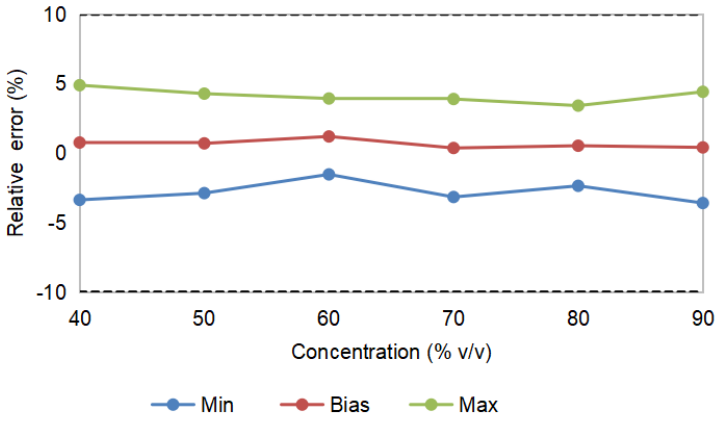

Figure 5. Accuracy profile of the developed quantitative method. For each concentration level, the minimum (min) and maximum (max) tolerance intervals are within the acceptance limits (±10%).

Applications

The developed qualitative and quantitative NIR methods were used to analyze 24 commercial disinfectants of different brands and batches. According to the DD-SIMCA analysis, 21 out of 24 samples were compliant, i.e had an ethanol content of between 60 and 80 % (Figure 6). The other samples (numbers 12, 13, and 16) were rejected by the model and therefore appeared to be of poor quality. The quantitative analysis with the PLS model showed that of the 21 samples identified as compliant by the DD-SIMCA model, 19 had an ethanol content higher than 60% v/v (between 67.0 and 80.9 % v/v) (Table 3). The percentage of stated content (ratio of the measured concentration to the nominal concentration stated on the sanitizer label) for these samples ranged from 90 to 110%, so they can be considered effectively compliant.

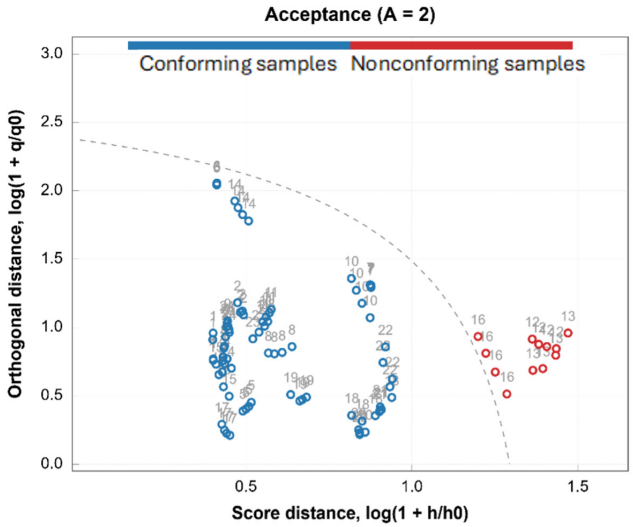

Figure 6. DD-SIMCA analysis of collected hand sanitizer samples. The dashed line delimits the acceptance area. Samples are deemed to belong to the target class (conforming samples) if they fall within the acceptance area.

For the two other samples (numbers 7 and 18) identified as compliant by the DD-SIMCA model, the measured ethanol content was 56.6% and 57.3% v/v, respectively. These concentrations near 60% v/v may explain the results obtained with the qualitative model, whose specificity compared to samples with 50% ethanol was 83%.

The ethanol content of the samples identified as non-compliant by the qualitative model (samples 12, 13, and 16) was found to be below 60% v/v (Table 3). Their stated content percentage was outside the 90-110% range; therefore, they were of poor-quality sanitizers. The comparison of the predicted ethanol concentrations of liquid samples (including samples 16 and 18) with those obtained with an alcoholmeter used as a reference showed that the relative errors were less than 4% (Table 3). This allowed us to confirm the reliability of the developed quantitative method. The results demonstrated the applicability of the developed chemometric models, based on a low-cost NIR spectrometer, as screening methods for analyzing alcohol-based hand sanitizers.

Conclusion

In this study, qualitative and quantitative methods using a low-cost near-infrared spectrometer for the quality control of alcohol-based liquid and gel sanitizers have been proposed. The qualitative method based on DD-SIMCA showed satisfactory results of sensitivity and specificity. The quantitative method based on PLS was fully validated using the accuracy profile methodology, which complies with ICH requirements for the validation of analytical methods. The methods developed and validated were applied to the analysis of various commercial disinfectants, enabling the detection of poor-quality products. The proposed NIR methods are fast, non-destructive, environmentally friendly, inexpensive, and reliable. Therefore, they are suitable for the quality control of hand sanitizers, particularly in resource-limited laboratories.

Table 3. Results of the quantitative analysis of collected hand sanitizers.

Sample Number	Dosage form	Nominal concentration (% v/v)	NIR measured concentration (% v/v)	Alcoholmeter measured concentration (% v/v)	Relative error (%)	Percentage of stated content (%)
01	Liquid	70	70.3	73	-3.7	100.4
02	Liquid	70	70.2	73	-3.8	100.3
03	Liquid	70	72.5	75	-3.3	103.6
04	Liquid	75	69.5	71	-2.1	91.9
05	Gel	70	72.5	NA	NA	103.6
06	Gel	70	70.6	NA	NA	98.2
07	**Gel**	**70**	**56.6**	**NA**	**NA**	**80.9**
08	Gel	70	63.6	NA	NA	90.8
09	Gel	70	72.7	NA	NA	103.9
10	Gel	70	69.0	NA	NA	98.6
11	Gel	70	72.8	NA	NA	106.1
12	**Gel**	**70**	**44.2**	**NA**	**NA**	**63.1**
13	**Gel**	**70**	**42.8**	**NA**	**NA**	**61.2**
14	Gel	80	72.2	NA	NA	94.4
15	Gel	70	67.6	NA	NA	95.7
16	**liquid**	**70**	**52.2**	**52**	**0.4**	**82.9**
17	Gel	75	70.2	NA	NA	93.6
18	**Liquid**	**80**	**57.3**	**55**	**4.2**	**70.3**
19	Liquid	80	80.9	81	-0.1	101.1
20	Liquid	80	78.0	82	-4.8	97.5
21	Liquid	80	79.5	82	-3.0	99.4
22	Liquid	80	79.9	79	1.1	99.9
23	Gel	70	67.5	NA	NA	96.4
24	Gel	70	66.8	NA	NA	95.4

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Disclosure statement

The authors declare no conflicts of interest.

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