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Development and Validation of a Near Infra-Red (NIR) Hand-held Spectrophotometric Method Using PCA Approaches and Chemometric Tools: Application for Qualitative and Quantitative Determination of Tadalafil Marketed in Kinshasa—D.R. Congo

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Abstract: A hand-held NIR spectrophotometric method was developed, validated, and applied for the determination of tadalafil in tablets. The aim of our work was to develop analytical methods based on vibrational techniques using low-cost portable equipment. Based on different chemometric modeling, we attempted to validate the method, which gave encouraging results from the principal component analysis (PCA), DD-SIMCA, and PLS modeling. Following this, we optimized the method using an appropriate experiment plan. For validation, we used the total error approach with acceptance limits set at ±10% with a risk level of 5%. The method showed that it was possible to perform both qualitative and quantitative analysis of pharmaceutical products using low-cost portable NIR systems with chemometric tools. The developed approach enabled the completion of the first step in implementing an NIR method for quality control of tadalafil-based drugs in the DRC. Validation difficulties of the PLS method resulted from the lack of information about inter-day serial variations of spectral responses. It would be interesting to extend the study to a larger calibration interval in order to correct uncertainties that may result from the variability observed under different conditions and to verify robustness. These are the limitations of this work, but the results are nevertheless very encouraging.

Keywords: substandard and falsified medicines; hand-held NIR; chemometric tools; development; validation

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1. Introduction

Globally, sub-standard and falsified medicines pose a serious risk to public health. Pharmaceutical falsification affects wealthy countries such as the United States, European countries, Australia, Japan, and others. This includes developing countries where falsified medicines are frequently distributed. According to the World Health Organization (WHO), any medication that has been fraudulently and purposefully mislabeled regarding its identity or source is considered falsified. Both branded and generic products are subject to falsification; examples of falsified products include those with the proper or incorrect active

ingredients, no active ingredients, insufficient active ingredients, or phony packaging [1]. Phosphodiesterase (PDE) 5 inhibitors for erectile dysfunction, such as tadalafil (Cialis), sildenafil (Viagra), and vardenafil (Levitra), are among the most popular categories of personal-import medications. In Japan, reports from 2011 and 2012 indicated that tadalafil that had been fabricated posed health risks. Compared to 2005, the number of counterfeit medications that failed customs inspection surged one hundredfold in 2015, with PDE 5 inhibitors accounting for a large portion of such medications. A collaborative survey conducted in 2016 by four producers and distributors of erectile dysfunction medications revealed that 40% of the test samples were fabricated. There have also been reports of tadalafil and other phosphodiesterase type 5 inhibitors that were not real from Europe, the US, Canada, and Australia [2–4]. These medications are frequently made in unregulated street labs that do not adhere to appropriate manufacturing practices. The effectiveness and safety of falsified medicines are extremely unreliable since their source is unknown, and no quality control is carried out. Ingredients can range from harmless components to toxic and dangerous elements. The number of medications that require testing has increased due to growing concerns about falsified products, which emphasizes the need for quick and low-cost preliminary screening techniques. The problem of fake medications makes it abundantly evident that analytical methods are required in order to identify these false products and differentiate them from real medications. Several analytical methods have already been documented in the literature and can be broadly categorized into two categories: spectroscopic and chromatographic methods. Large quantities of these products arrive on the European market, which requires the development of simple and rapid methods to help customs officers detect them; this is the case with the near-infrared technique [1,2,5].

In their Roadmap for Supply Chain Security, the Asia Pacific Economic Cooperation stated that it is critical to create strategies and procedures for identifying and stopping the production of falsified products. However, the advancement of detection techniques has been sluggish, and no trustworthy techniques have surfaced. Pharmaceutical analysis has traditionally relied on high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC), which are capable of precisely identifying and measuring active pharmaceutical ingredients (API). Chromatographic techniques, particularly liquid chromatography coupled with mass spectrometry (LC-MS, HPLC-MS, UPLC-MS), are widely used because they produce consistent results. Nevertheless, there are a number of disadvantages to their use, such as the time-consuming nature of sample preparation and analysis, the high cost of the equipment, and the requirement for a skilled analyst. Furthermore, chromatography-based techniques may be harmful to the environment, primarily because solvents are used in the sample preparation and chromatographic separation processes [6–9]. Thus, several alternative methods are used to verify the identity of batches of starting materials in accordance with the Pharmaceutical Inspection Convention and Pharmaceutical Inspection Co-operation Scheme, such as portable spectroscopic analyzers. Spectroscopic techniques, including near-infrared (NIR), Fourier transform IR, nuclear magnetic resonance (NMR), and Raman spectroscopy, have been developed as quick, non-destructive methods of analysis. The main non-destructive technique used in the pharmaceutical industry that does not require sample preparation is NIR spectroscopy [10,11]. One such technique is gas chromatography-mass spectrometry (GC–MS). They do not cause destruction [12]. Since longer wavelengths of fluorescence are generally acknowledged to result in lower levels of external interference, attempts to alter the fluorescence structure (such as by creating probes with near-infrared emission) may be successful. Furthermore, a ratio metric pattern, which simultaneously monitors the fluorescence intensity of two wavelengths, can effectively eliminate external interference and improve detection sensitivity. However, vibrational spectroscopy is a preferred detection and quantification technology in the crop industry and can address some of the limitations of the aforementioned methods when combined with multivariate statistical analysis techniques (chemometrics). In particular, near-infrared (NIR) spectroscopy showed that it could accurately quantify a wide range of elements and compounds [2,13,14]. Infrared spectroscopy has shown

potential as a tool for control in different environments [15–18], and it also demonstrated benefits for non-invasive and immediate detection, which allowed the technique to gain acceptance and a wide range of use in industries, including biomedicine and more. It also performs well when applied to other tasks such as adulteration detection, drug forgery identification, crop quality testing, etc. Miniaturized NIR spectrometers are becoming more and more common due to advancements in electronic technologies and microelectromechanical systems; portable models have been commercialized up to this point. Portable NIR spectrometers are very popular because of their flexibility, and their range of uses has grown from traditional laboratory settings to spot inspection [19–22]. Combined with chemometric tools, this technique can be more performant in evaluating products from any matrix, with possible online analytical applications. One of its advantages is the greater penetration of radiation in the sample; this makes it possible to analyze the thicker solid samples by reflectance to obtain more representative information [23–26].

Several measurement modes can be used depending on the sample shape, reflection, transmission, or transflection [13]. It is important to emphasize that the development of NIR methods for the quantification of solid dosage forms (tablets) presents major advantages in that they allow measurements to be carried out with little or no prior treatment of the samples. However, these methods present several challenges due to the influence of physical properties on the spectra. Due to the presence of a variety of excipients and dosage forms, these methods generally require the analyst to develop specific predictive models for each formulation. In order to solve this problem, this is a continuation of the work of P.H. Ciza et al. [13,14], except that here, for the first time, we tested a non-soluble compound and used HCl 0.1 N as solvent.

Therefore, this research aimed to develop and validate an analytical method using near-infrared for qualitative and quantitative determination of tadalafil marketed in Kinshasa and for its market surveillance in D.R. Congo with the use of chemometric tools combining PCA application and data pretreating. After development, the PLS method was validated and applied for quantitative application.

2. Materials and Methods

2.1. Chemicals

2.1.1. Pharmaceutical Substances and Brands

Tadalafil (Standard, 99.3%) was from ZM Technologies Limited, Liverpool, England. Magnesium stearate, Monohydrate lactose, cellulose microcrystalline, cross carmelose, and tadalafil samples were from Kim Pharma, Kinshasa, D.R. Congo. For reasons of confidentiality, the Tadalafil brand is coded as MCC, MMP, MXX, and MTT. The Table 1 groups all the analyzed brands giving in extension for each of them the applicant name, brand name, manufacturer, origin country, the authorization number, the registration date, validity period and expiration date.

a. **Brand MCC**

Dark yellow, biconvex, diamond-shaped, film-coated tablets in rectangular blister packs, each containing two tablets. Each blister is packaged in a yellow, green, and white cardboard box, with two blister packs in the latter. Each box also includes a leaflet containing composition, pharmacological properties, indication, contraindication, warnings, dosage, side effects, drug interactions, overdose, presentation, and storage.

Dosage: 20 mg
Manufacturer: Eli Lilly
Preemption date: June 2025
Batch number: 09968

b. **Brand MTT**

Dark pink, rounded, lozenge-shaped, film-coated tablet contained in a rectangular blister pack. Each blister pack contains four tablets. Each blister is packaged in a blue-gold cardboard box containing a leaflet with composition, pharmacological properties,

indication, contraindication, warning, dosage, side effects, drug interactions, overdose, presentation, and storage.

Dosage: 20 mg
Manufacturer: Lincoln Pharmaceuticals Ltd
Preemption date: January 2024
Batch number: NX 1001

c. **Brand MXX**

Dosage: 20 mg
Manufacturer: Kim Pharma
Preemption date: July 2025
Batch number: XT-11

d. **Brand MMP**

Pink, biconvex, diamond-shaped, film-coated tablet contained in a rectangular blister pack. Each blister pack contains two tablets. Each blister is packaged in a black, yellow cardboard box containing a leaflet with composition, pharmacological properties, indication, contraindication, warning, dosage, side effects, drug interactions, overdose, presentation, and storage.

Dosage: 10 mg
Manufacturer: Paloma
Preemption date: September 2025
Batch number: M-03

Table 1. Different tadalafil brand information.

Applicant	Product Name	Manufacturer	Origin Country	Authorization N°	Registration Date	Validity (Year)	Preemption
KIM PHARMA	X-1 20 mg, capsule, bxe of 2.	ETS KIM PHARMA	Congo	MS.1253/10/05/DGM/ 0108/2016	May 2016	5	May 2021
ELI LILLY NEDERLAND	Cialis 20 mg	Eli lilly Nederland	The Netherlands	EU/1/02/237/006	Nov 2012	10	Nov 2022
	Mon plaisir	-	-	-	-	-	-
	Tadalanique	-	-	-	-	-	-

2.1.2. Solvents and Reagents

Analytical grade methanol used for sample preparation was obtained from Kim Pharma-Kinshasa, D.R. Congo. 37% analytical grade hydrochloric acid was used to dissolve components provided by VWR in Leuven, Belgium. The demineralized water was obtained from the faculty MilliQ Plus 185 (Waters, UK) system and purchased from Molshein in France and Acetone from Merck in Germany.

2.2. Near Infrared Instrumentations

Two NIR spectrophotometric devices were used in our research, both dispersive portable NIR spectrophotometer systems, namely NIR-S-G1, Innospectra Corp branded (Hsinchu, Taiwan), and NIR-M-T1, Innospectra Corp branded (Hsinchu, Taiwan). Measurements were conducted in two modes (reflection and transmission modes). The NIR devices were checked for their wavenumbers accuracy, and the X-axis was recalibrated as necessary as possible to ensure a lower difference (± 1 cm^{-1}).

2.3. Glassware and Small Equipment

An Eppendorf (5 and 50 mL), a volumetric pipette (1 mL), an analytical balance SV gram precision branded beaker, a bowl, gloves, and paper towels were used to facilitate experiments.

2.4. Preparation of Solutions

2.4.1. NIR Calibration and Validation Standards

Calibration and validation samples were prepared by dissolving reference tadalafil in methanol. Tadalafil standards were prepared in the presence of a sufficient quantity of mixing excipients to mimic tablets using gravimetric data as a reference. Three independent sample runs were performed for calibration and validation purposes with five concentration levels (5, 7.5, 10, 12.5, and 15 mg/mL tadalafil) in which the target concentration for the samples was 10 mg/mL.

Before analysis, tadalafil standard solutions containing excipients were shaken by hand for a maximum of 5 min, then left to settle for 10 min. The supernatant was finally filtered through a 4.5 μm filter. All calibration and validation solutions were prepared in triplicate for each concentration level and measured three times. For validation calculations, the three predictions were averaged per sample.

For qualitative model validation, solutions containing different brands of Tadalafil tablets were prepared at the target concentration of 10 mg/mL. In addition, a placebo solution with the excipient mixture was prepared at the target level.

2.4.2. Sample Preparation

Test solutions were prepared by simple dissolution or dilution of the unit dosage form with methanol to obtain a final solution of 1% w/v of the declared strength. The suspensions obtained from the tablet test sample were then shaken by hand for a maximum of 5 min, allowed to stand for 10 min, and passed through a 4.5 μm filter as for Tadalafil standards.

2.4.3. Sample Essays and Spectral Measurements

The target concentration of 10 mg/mL was obtained by dilution in HCl 0.1 N for each collected sample of Tadalafil. This was performed for every single sample, and each sample solution was filtered to obtain three measurements. For all the tests, predictions were averaged for n equals 3.

Tadalafil spectral measurements were performed using a NIR-M-T1-A device in transmission (sample in solution). Chemometric techniques and the spectral range were helpful for optimization and allowed the building of PLS models. Table 2 describes how the NIR spectra were processed, including all applied models to smooth, filter, and remove the noise.

Table 2. Selected model parameters.

Metric	NIR-M-T1
Spectral range	1530–1642 nm
Preprocessing	SG(1,2,15) + SNV + MC
PC	3
A	0.001
Sn (VAL)%	97.7
Sp (Cialis)%	33.3
Sp (placebo)%	100.0

SG: Savitzky-Golay (derivative, polynomial order, window size). MC: Mean centering. SNV: Standard Normal Variable. Sn: Sensitivity. Sp: Specificity.

3. Results and Discussion

3.1. Data Acquisition and Chemometric Pretreating

Chemometric calculations and analyses were performed in MATLAB (R2018a) (The Mathworks, Inc., Natick, MA, USA). For PLS models, the PLS Toolbox v.4.11 (EigenvectorResearch, Inc., Wenatchee, WA, USA) was used.

To reduce variability and improve chemical spectral characteristics, the raw NIR spectra were pre-processed.

Tadalafil spectra recorded with the NIR-S-G1 device in reflection (solid-state) and with NIR-M-T1 in transmission (dissolved in methanol) are shown in Figures 1 and 2, respectively. Specific spectral features of Tadalafil are present in the spectral range where the absorption of O-H bonds in methanol is low (between 1500 and 1640 nm). For the spectrum of tadalafil in methanol, no difference is observable to the naked eye. However, chemometric techniques can detect very small differences in absorption. Figure 3 shows comparative spectra recorded using NIR-S-T1 in transmission during determinations of tadalafil brand samples, tadalafil standard, matrix (made of excipients mixture), and the blank (methanol).

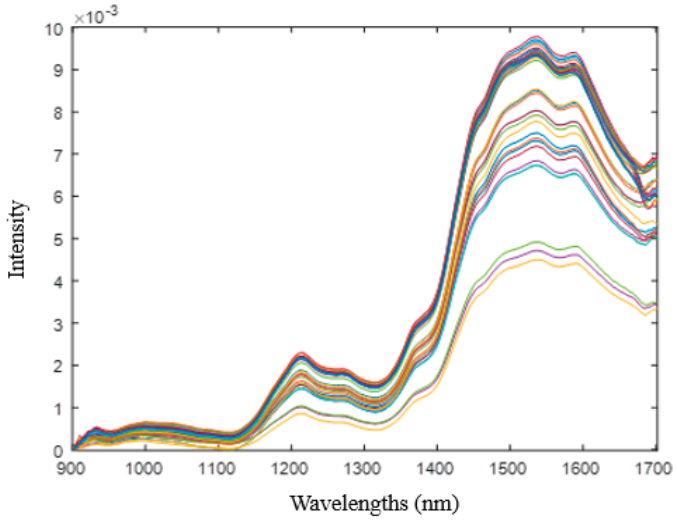

Figure 1. Tadalafil spectra were recorded with the NIR-S-G1 device in reflection (with pink curve representing the blank, capri blue curve representing excipients, blue color for standard and all the other colors as different tadalafil brands used for testing).

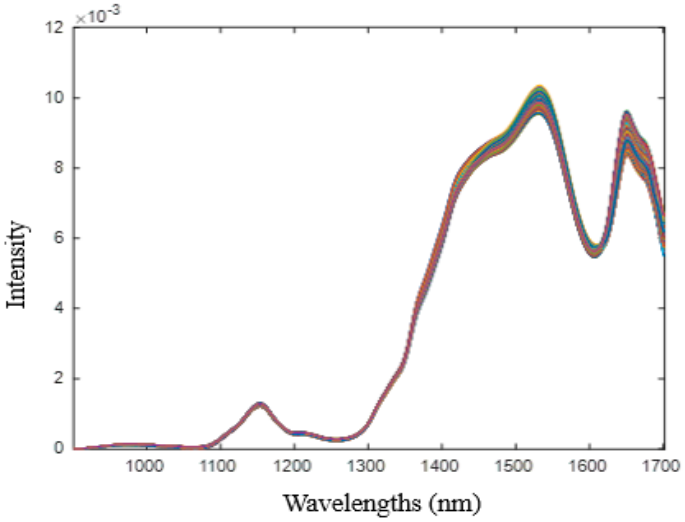

Figure 2. Transmission spectra of NIR-M-T1 recorded Tadalafil (with pink curve representing the blank, capri blue curve representing excipients, blue color for standard and all the other colors as different tadalafil brands used for testing)

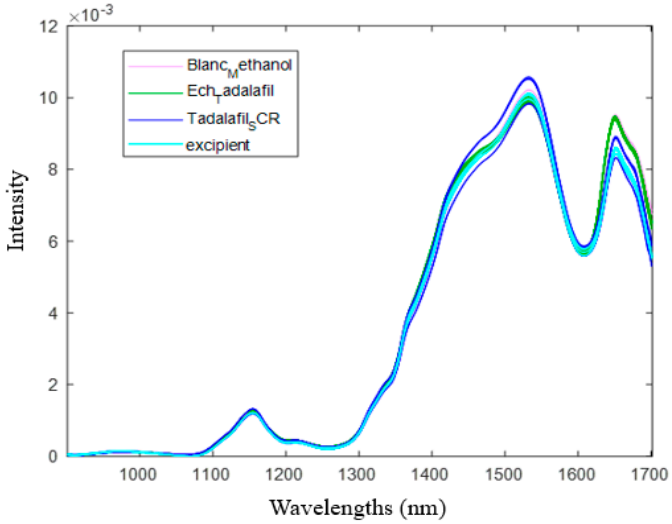

Figure 3. Spectra of tadalafil tablet samples (green curve), matrix (excipient mixture, in capri blue curve), blank (represented by the pink curve), and tadalafil SCR (blue curve) recorded NIR-M-T1 in transmission.

3.2. Chemometric Data Analysis

3.2.1. PCA Models (Principal Component Analysis)

The PLS models were obtained using tadalafil-branded sample spectra and were compared once obtained from the two NIR Spectrometers. As can be seen in Figure 4, there is considerable variability between tadalafil tablet samples (green) and tadalafil standard samples (blue). When the results are compared between the different samples, there is a significant difference in terms of spectral signature.

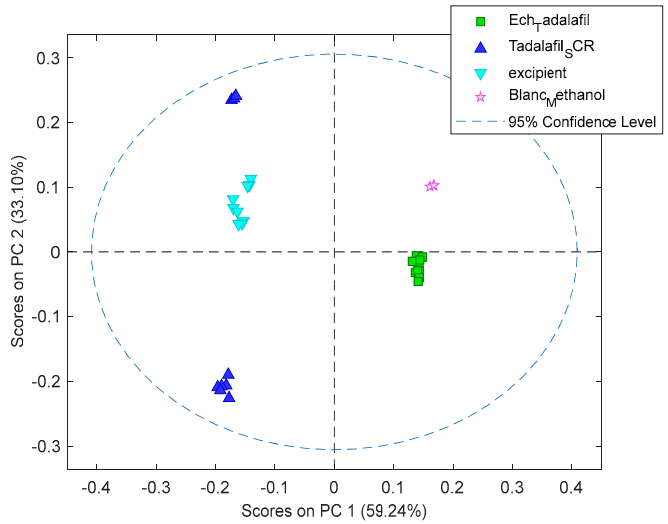

Figure 4. Principal component analysis of spectral data obtained. Score diagram representing the variability between different samples.

3.2.2. DD-SIMCA Models

Due to the nature of NIR spectra (broad, highly overlapping bands), it is recommended to use a qualitative model before the PLS regression model.

In this study, Data-Driven Soft Independent Modelling Class Analogy (DD-SIMCA) was used to build qualitative identification models. DD-SIMCA is based on the construction of a principal component analysis (PCA) model of the target class, which, in our case, corresponds to the calibration spectra. In this way, the score distance (SD) and orthogonal

distance (OD) can be calculated for each future spectrum, making it possible to determine, at a given confidence level, the acceptance zone for authentication of a specific brand. In our case, DD-SIMCA models were also used to distinguish X-1 Cialis from related (tadalafil-based) formulations but with different matrices.

A placebo solution (excipient and blank) was also projected onto the DD-SIMCA model to ensure that X-1's excipients did not interfere with the qualitative models.

Sensitivity was assessed using the new set (tadalafil validation set) on the DD-SIMCA models, using the following formula:

$$Sensitivity\ (\%) = \frac{True\ Positive}{Positive} \times 100 \quad (1)$$

$$Specificity = \frac{True\ Negative}{Negative} \times 100 \quad (2)$$

As a first validation criterion, the specificity of the method must be demonstrated. It is generally good practice to build a qualitative model before the quantitative one. This ensures that only spectra similar to those used for calibration are projected onto the regression model.

As can be seen, visual spectral correlation cannot be a reliable method of selectivity. Due to the very small differences between spectra, pre-processing methods using Savitzky-Golay derivatives improve spectral characteristics and facilitate discrimination of different APIs in SIMCA models. In addition, chemometric methods were used to help detect spectral dissimilarities between these samples. A single-class classification model was developed using the DD-SIMCA approach. The model parameters selected are listed in Table 1, together with the associated sensitivity and specificity.

DD-SIMCA acceptance plots for calibration and validation data are shown in Figures 5 and 6, while Figures 7 and 8 show DD-SIMCA acceptance plots for X-1 and Cialis tablet data. The DD-SIMCA (Figures 5–9) models applied to NIR data enabled perfect recognition of X-1 samples and perfect discrimination of placebo and, in part, Cialis samples. This confirms its applicability to systematically reject non-X-1 Tadalafil samples prior to quantitative analysis.

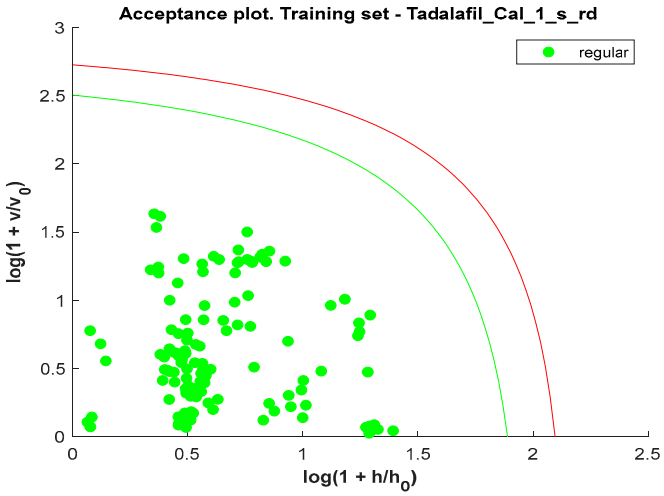

Figure 5. DD-SIMCA acceptance diagram for calibration samples. Samples below the threshold (green col-ored dots) are considered as standards of the target class and were used to build the model (The green dots reflect samples that are considered regular in reference to the calibration model and so authentic for the analysis performed. The green line defines the acceptance zone, within which the result can be deemed good and authentic, while the red line indicates the impassable boundary beyond which the product would be inauthentic, and the gap between the two lines is considered the warning zone).

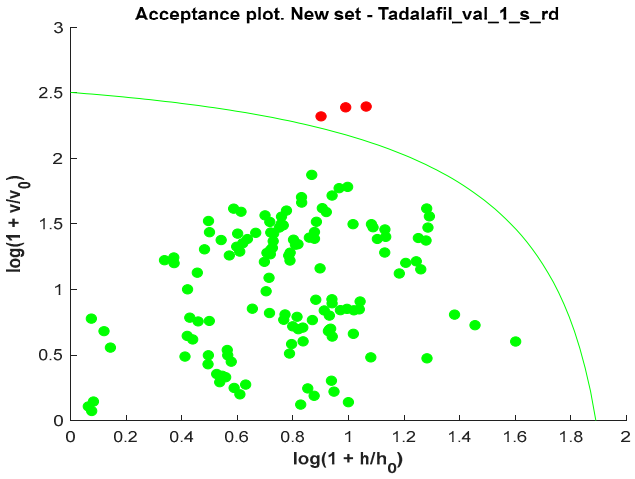

Figure 6. DD-SIMCA acceptance diagram for validation samples. Samples below the threshold (green) are considered as belonging to the target class. Samples above the threshold (red) are considered outliers.

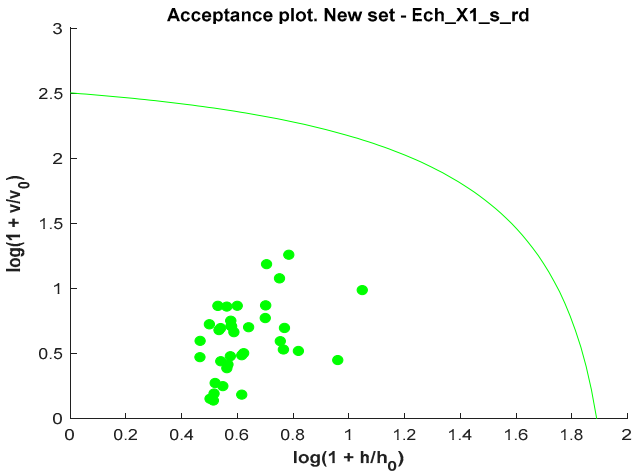

Figure 7. DD-SIMCA acceptance diagram for X-1 samples. All samples below the threshold (green color) are considered authentic.

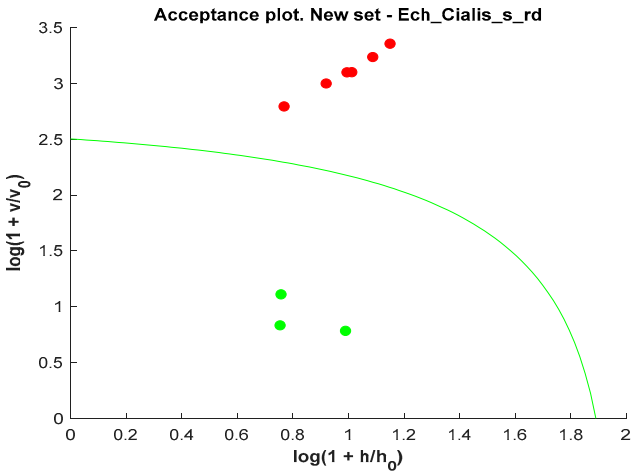

Figure 8. DD-SIMCA acceptance chart for Cialis samples. Only a third of the samples below the threshold (green color) are considered authentic.

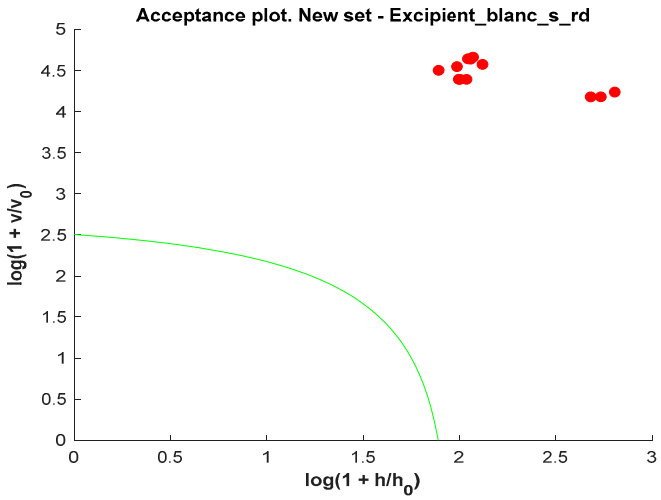

Figure 9. DD-SIMCA acceptance diagram for matrix and blank. All samples above the threshold (red color dots) are considered outliers as the green line represents the acceptability region limitation.

Nevertheless, performance showed a sensitivity of 97.7% for tadalafil validation samples, a specificity of 100% for matrix, and a specificity of 33.3% for Cialis.

3.3. PLS Analysis

Several PLS models have been built using different preprocessing methods or combinations of them, taking into account different numbers of latent variables (see Figures 10 and 11).

Selecting an appropriate number of latent variables avoids under- or over-fitting the model.

The PLS Toolbox model optimizer was used to preselect spectral ranges, preprocess, and a number of latent variables, using RMSEP as a quality criterion. The last few models selected were compared based on accuracy profiles reflecting the method's current use.

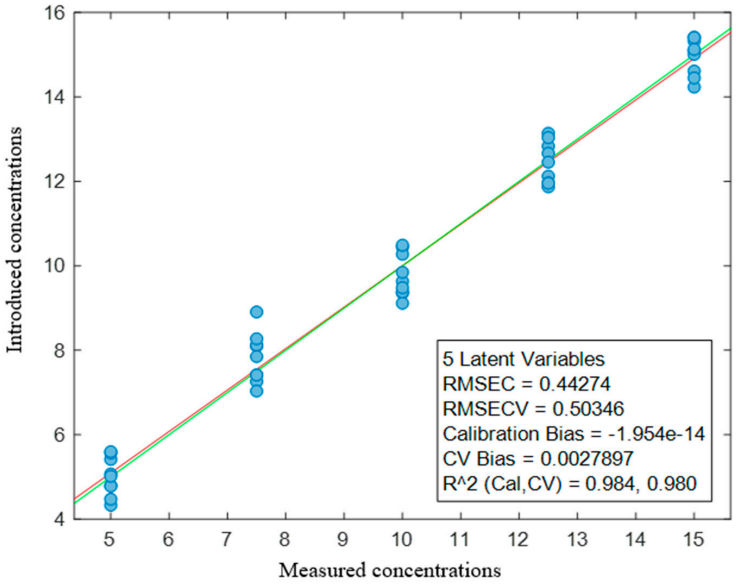

Figure 10. Graph showing the characteristics of the PLS calibration model for NIR data (The blue dots repre-sent the number of analysis repetitions on each level of concentration as they progress from one to the next, as well as the dispersion of the responses obtained each time; the green line represents what would be a better characteristic of the calibration model and what would be desirable to obtain with the samples for acceptance of the analysis results; and the red line corresponds exactly to the inverse of what the green line provides as information according to this calibration model).

The wide dispersion of the relative error of different concentration levels can be explained by the fact that the matrix used contained excipients that could pass into the methanolic solution (due to the higher excipient/API ratio). This has a random impact on the amount of tadalafil present in the solution after the filtration step. After being developed, the PLS model was tested for its predictive ability on spectral data taken under multi-source environmental conditions (change in temperature and relative humidity). Unfortunately, a bias was observed when the developed model was used to predict validation samples measured under different conditions (see Figure 6).

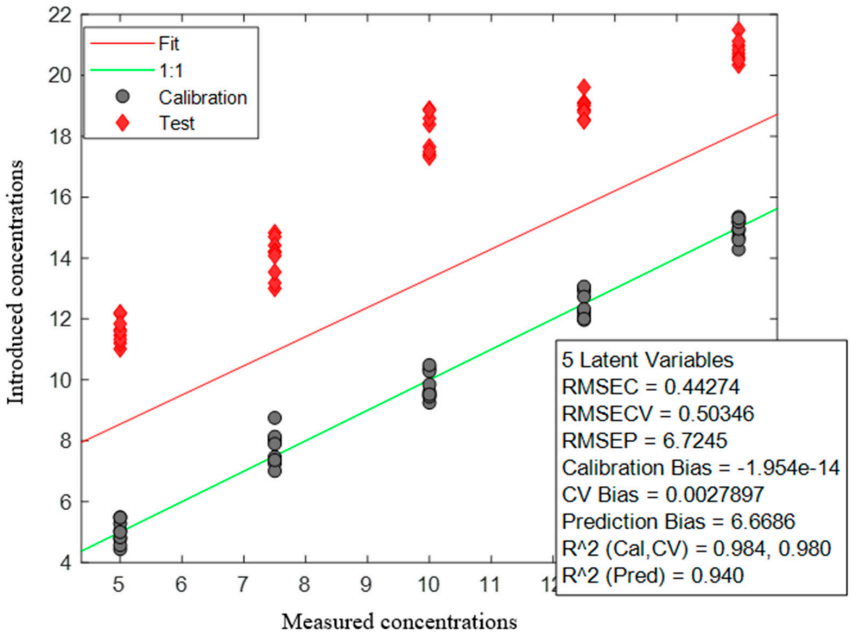

Figure 11. Graph showing the characteristics of the PLS calibration model developed for the prediction of validation data.

When looking at the score graphs, the validation data are considered to be outliers in relation to the model and, unfortunately, cannot be analyzed directly without correction (Figure 12).

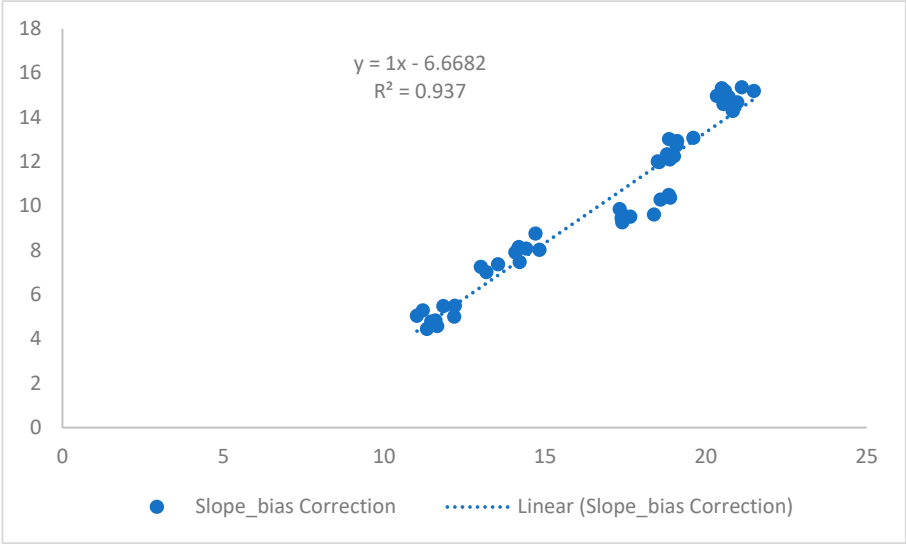

Figure 12. Characteristics of the linear Y model.

The slope-bias correction SBC performed a linear-univariate model study between the predicted data and the standard data; then the linear model (taking into account the slope and y-intercept) was used to correct the predicted data using the following equation:

$$Y_{(val)Corr} = Bias + Slope\ Y_{(val)}$$

In some cases, this may be one of the alternative solutions for compensating for NIR spectral variations that may result from instrumental or environmental variability, for example, when samples and/or spectral measurements are significantly affected by changes in temperature, relative humidity, or instrumental variability. NIR-M-T1 portable instruments are still in the development phase and do not have waterproof shells to protect the system from temperature and/or humidity variations. As a result, particular attention needs to be paid to their use in tropical zones, where there are strong seasonal variations in temperature and relative humidity.

For SBC, the values of R^2 (coefficient of determination), (the slope of the linear fit), and y-intercept, using predicted versus calibration data, are presented in the linear equations in Figure 8. The SBC corrected the bias observed when predicting standard tadalafil samples measured under different environmental conditions. The corrected predicted data enabled the model to be successfully validated in the tadalafil concentration range.

However, it would be interesting to test under different environmental conditions and check when convergence is reached, indicating the optimal condition to include in the overall modeling. Another approach could be global modeling. This approach consists of adding the new variability resulting from the new data taken under different environmental conditions to the calibration set.

Validation

The NIR predictive model was validated using the total error approach with acceptance limits of ±10% and a risk level of 5%.

All validation calculations were performed with E-noval 4.0b (PharmalexBelgium, Mont-saint-Guibert, Belgium).

Experiment plan

Validation standards are samples reconstituted in the matrix containing a known concentration and whose value is considered true by consensus.

Table 3 shows the number of validation standards per concentration level, the concentration levels considered, and the different series performed.

Table 3. Experiment plan.

Series	Concentration Level (mg/mL)	No of Independent Replicates
1	1.0	3
1	2.0	3
1	3.0	3
1	4.0	3
1	5.0	3
2	1.0	3
2	2.0	3
2	3.0	3
2	4.0	3
2	5.0	3
3	1.0	3

Table 3. *Cont.*

Series	Concentration Level (mg/mL)	No of Independent Replicates
3	2.0	3
3	3.0	3
3	4.0	3
3	5.0	3

Total number of observations: 45.

3.4. Validation Criteria Study

3.4.1. Trueness

Trueness expresses the closeness of agreement between the mean value obtained from a large series of test results and an accepted reference value. Trueness gives an indication of systematic errors.

As shown in Table 4, trueness is expressed in terms of absolute bias (mg/mL), relative bias (%), or recovery rate (%) for each concentration level of the validation standards.

Table 4. The trueness.

Concentration Level (mg/mL)	Mean of Entered Concentrations (mg/mL)	Mean of Results (mg/mL)	Absolute Bias (mg/mL)	Relative Bias (%)	Recovery Rate (%)	95% Confidence Interval Recoveries (%)
1.00	5.00	4.94	−0.06	−1.24	98.76	[92.48, 105.00]
2.00	7.50	7.69	0.19	2.51	102.50	[99.64, 105.40]
3.00	10.00	10.35	0.35	3.49	103.50	[101.70, 105.20]
4.00	12.50	12.55	0.05	0.42	100.40	[99.32, 101.50]
5.00	15.00	14.58	−0.42	−2.83	97.17	[95.57, 98.77]

3.4.2. Precision

Precision expresses the closeness of agreement between a series of measurements taken from multiple replicates of the same homogeneous sample under prescribed conditions. It provides information on random error and is assessed at two levels: repeatability and intermediate precision.

As shown in Table 5, precision (repeatability and intermediate precision) can be expressed in terms of standard deviation (SD) and coefficient of variation (CV).

Table 5. Repeatability and relative intermediate precision.

Concentration Level (mg/mL)	Mean of Entered Concentrations (mg/mL)	Repeatability		Intermediate Precision	
		SD	CV%	SD	CV%
1.00	5.00	0.20	3.98	0.46	9.15
2.00	7.50	0.20	2.67	0.30	4.02
3.00	10.00	0.23	2.29	0.23	2.29
4.00	12.50	0.15	1.20	0.18	1.49
5.00	15.00	0.12	0.80	0.35	2.36

CVs in % of Repeatability and Intermediate precision were obtained by dividing the standard deviation (SD) obtained by the corresponding mean of the introduced concentrations.

The precision of this method was assessed at two levels: repeatability and intermediate precision. The coefficient of variation (CV) was used as an expression of this precision.

For good method fidelity, the percentage CV must not exceed 2.000 at all concentration levels studied. This explains the good precision only at levels 4.0 and 5.0; repeatability and inter-day intermediate precision are very good considering only these two levels.

3.4.3. Accuracy

Accuracy expresses the closeness of agreement between the test result and the reference value accepted as such, also known as the "conventionally true value". Accuracy takes into account the total error, i.e., the systematic error and the random error associated with the result. Consequently, accuracy is the sum of trueness and precision. It is estimated from the accuracy profile shown in Figure 13.

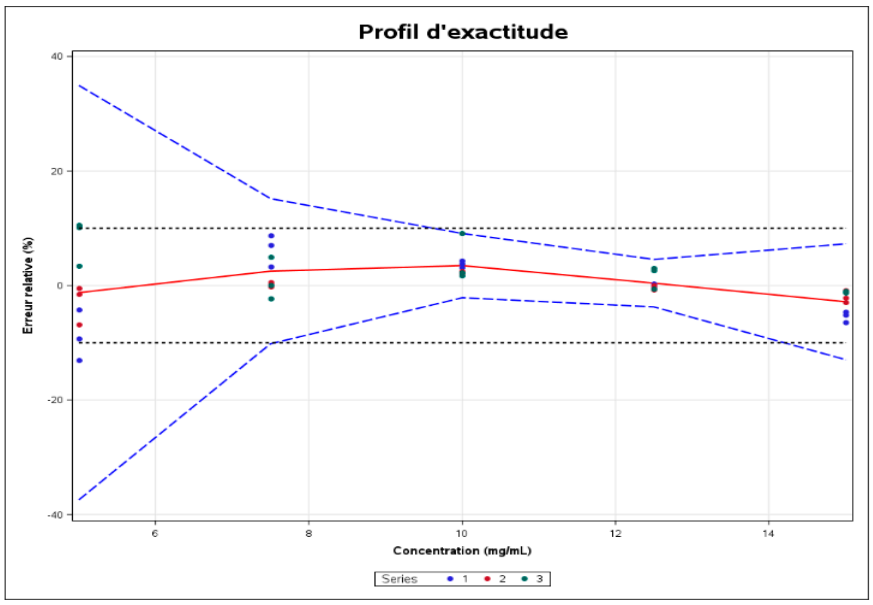

Figure 13. Accuracy profile.

Acceptance limits have been set at ±10%, in line with the objective of the analytical procedure (USP).

The solid red line represents the relative bias, the dashed blue lines define the limits of the tolerance interval expected at the beta level, and the dashed black lines are the acceptance limits. The dots represent the relative error of the results and are plotted against their target concentrations.

3.4.4. Linearity

The linearity of an analytical method is its ability, within a certain assay interval, to obtain results directly proportional to the analyte concentration in the sample. A linear regression model (see Figure 14) was fitted to the results calculated as a function of the concentrations introduced in order to obtain the following equation:

$$Y = 0.3650 + 0.9655\ X$$

where Y = results (mg/mL)

And X = concentrations introduced (mg/mL)

The coefficient of determination (r2) is 0.9890.

The correlation coefficient (r) is 0.9945.

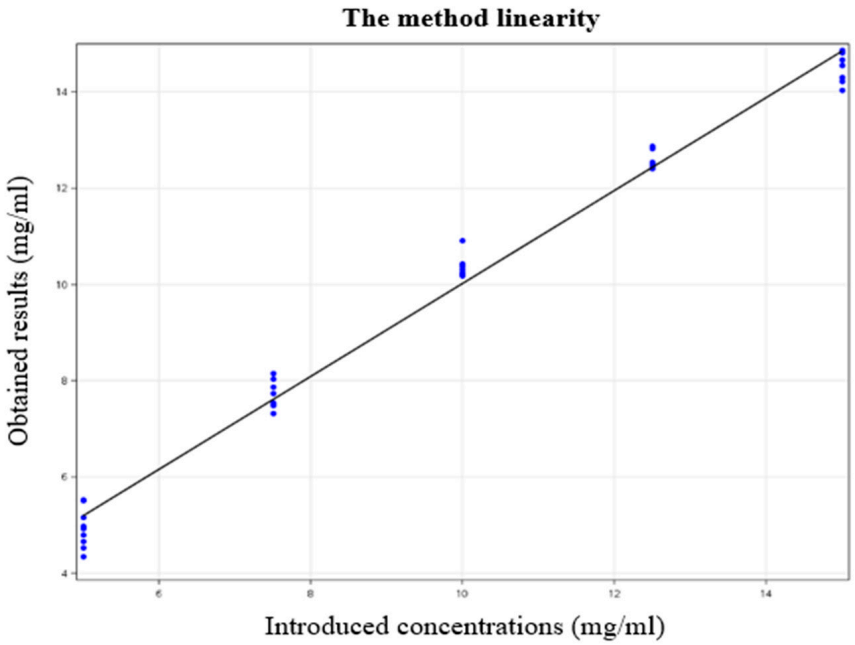

Figure 14. Relationship between introduced concentrations and results (The solid black line depicts the linea-rity of the relationship between different concentrations as they progress from one level to the next, as well as the number of analysis repetitions per level, whereas the blue dots represent the various concentration levels, with the number of dots corresponding to the number of repetitions).

The solid black line is the identity line ($Y = X$). The limits represented by the dashed blue lines on the graph correspond to the linearity profile, the blue dots represent the various concentration levels, with the number of dots corresponding to the number of repetitions and dotted black line represents the dispersion limit from the lower to the upper concentration values (Figure 15), i.e., the "beta-expectation" tolerance limits expressed in absolute values.

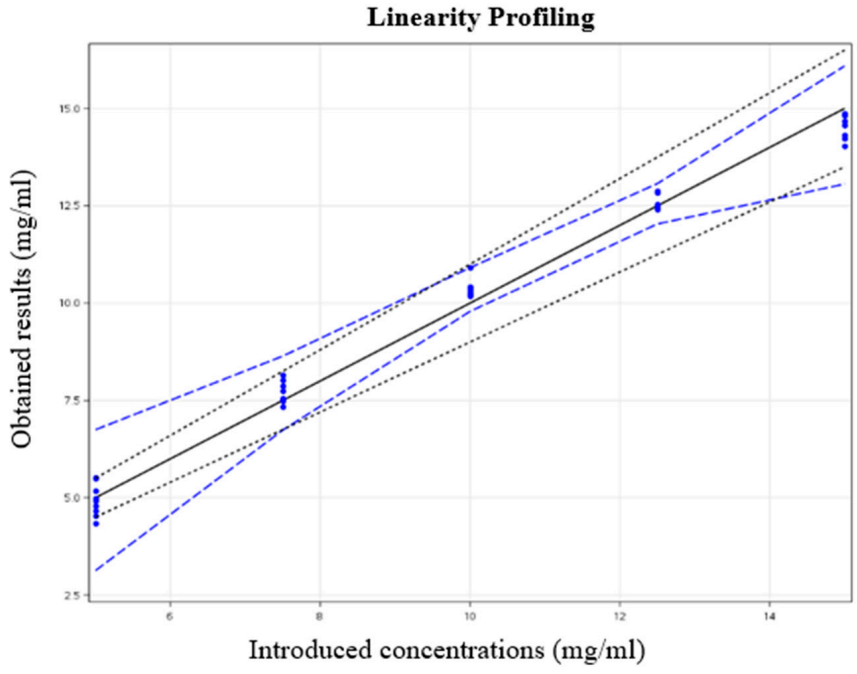

Figure 15. Linearity profile graph of acceptability.

In order to demonstrate the linearity of the method, the approach based on the expected tolerance interval at the beta level, expressed as an absolute value, can be used and is illustrated in the previous figure. As the figure shows, the linearity of the method is not exactly valid. This is due to the fact that R^2 (0.989) is not close to 1. In this case, we say that there is no good agreement between the concentrations introduced and the concentrations calculated for certain levels.

3.5. Method Application to the Drug Determination

It is important to remember that routine application is the final phase in the life cycle of an analytical procedure. As the developed method is not validated, it is important to cover all environmental variability and revalidate the method before considering its routine use. Nevertheless, a preliminary test was carried out on samples of X-1 tablets (Tadalafil 20 mg). Test solutions were prepared by simply dissolving the unit dosage form with aqueous methanol solution to obtain a final solution of 1% w/v of the declared content. The resulting test tablet sample suspensions were then shaken by hand for a maximum of 5 min and then allowed to stand for 10 min before passing through a 4.5 µm filter as per the tadalafil standards. The analytical results are presented in the Table 6 showed below and including for each sample the batches (for those where they were available, predicted concentration, obtained concentration and the content in percentage).

Table 6. Analytical results.

Samples	Batches	Predicted Conc. (mg/mL)	Corrected Conc. (mg/mL)	Content%
1	XT-11	17.24	10.57	105.75
2	XT-11	17.42	10.75	107.54
3	XT-11	16.75	10.10	100.77
4		17.98	11.31	113.13
5		17.24	10.57	105.72
6		17.65	10.98	109.83
7		17.84	11.17	111.66
8		17.67	11.00	110.01
9		17.54	10.87	108.72

Target concentration: 10 mg/mL.

4. Conclusions

Hand-held spectrophotometers have now become essential tools for quality control of pharmaceutical products, particularly for the field detection of substandard and/or falsified drugs. The aim of our work was to contribute to the development of analytical methods based on vibrational techniques and using low-cost portable equipment. Based on the results of our research, we can conclude that the developed method has shown that it is possible to perform both qualitative and quantitative analysis of pharmaceutical products using low-cost portable PIR systems combined with chemometric tools.

Difficulties in validating the PLS method were encountered due to the lack of information on variations in spectral responses between the different series prepared on different days. However, it would be interesting to extend this study to a larger number of calibration data in order to correct measurement uncertainties that may result from the variability observed under different environmental conditions, and to verify its robustness. These are the limitations of this work, but the results are nevertheless very encouraging.

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