Talanta Open 3 (2021) 100025

Contents lists available at ScienceDirect

Talanta Open

journal homepage: www.elsevier.com/locate/talo

Application of NIR handheld transmission spectroscopy and chemometrics to assess the quality of locally produced antimalarial medicines in the Democratic Republic of Congo

P.H. Ciza[a,b], P-Y. Sacre[a,*], M.R. Kanyonyo[c], C.T. Waffo[a,d], M.A. Borive[e], L. Coïc[a], J.K. Mbinze[b], Ph. Hubert[a], E. Ziemons[a], R.D. Marini[a,e]

[a] University of Liege (ULiege), CIRM, ViBra-Sante Hub, Department of Pharmacy, Laboratory of Pharmaceutical Analytical Chemistry, Liege, Belgium
[b] University of Kinshasa, Faculty of Pharmaceutical Sciences, LACOMEDA, Lemba, 212 Kinshasa XI, D.R. Congo
[c] Pharmakina, Agro-industrial and pharmaceutical enterprise, Bukavu, South Kivu, D.R. Congo
[d] University of Yaoundé I, Faculty of Medicine and Biomedical Sciences and National Drug Control and Valuation (LANACOME), Cameroon
[e] University of Kisangani, Department of Pharmacy, Kisangani, D.R. Congo

A B S T R A C T

In recent decades, more than 15% of the antimalarials marketed in low- and middle-income countries have been of poor quality, in which quinoline derivatives and quinine-based formulations account for 21%.

Near infrared spectroscopy (NIR) was chosen for its fast and inexpensive test properties as well as the ability of using handheld devices to monitor drugs directly on the field. Data driven - soft independent modeling of class analogy (DD-SIMCA) and partial least squares (PLS) regression models were developed for qualitative and quantitative purpose, respectively. The specificity and selectivity tests were performed using the DD-SIMCA models on the placebo, the quinidine and cinchonine standard samples. Then, PLS regression methods have been developed and validated for the quality control of quinine dosage forms manufactured by a major local manufacturer in the Democratic Republic of Congo (DRC).

Calibration and validation samples were prepared by dissolving quinine sulfate / quinine hydrochloride in the presence of excipients in HCl 1 M. The opportunity to work with dissolved quinine with a cheap and readily available medium in low- and middle-income countries allowed analysis of different pharmaceutical forms (oral drops, solutions for injection and tablets) with the same regression model. DD-SIMCA models have demonstrated, for both equipment, perfect authentication of quinine and good discrimination of the two alkaloids close to quinine namely cinchonine and quinidine.

The NIR PLS regression models were successfully validated using the total error approach with acceptance limits set at ± 10% with a risk level of 5%. The predictive performance of the methods developed was tested in terms of robustness.

1. Introduction

Large-scale production of pharmaceutical products generally involves several processes such as weighing, mixing, granulation, compression and requires as continuous real-time quality assurance a quality control throughout the process due to batch variability and regulatory constraints in manufacturing [1]. To this end, guidance from several regulatory authorities, such as the US Food and Drug Administration (FDA), under the Process Analysis Technology (PAT) initiative, encourages the use of innovative risk-based approaches to ensure product quality and performance [2–6]. This innovation of the pharmaceutical industry in low- and middle-income countries is generally considered essential to ensure the production of better quality and uniformity of the product [7].

Post-marketing quality control of medicines is also very important to ensure that only high quality medicines reach the patients. According to the recent definition of the World Health Organization (WHO), falsified medicines are medicines that deliberately/fraudulently misrepresent their identity, composition or source. Substandard also called "out-of-specification" medicines, are authorized medicines that fail to meet either their quality standards or specifications, or both [8]. This phenomenon poses a threat to public health and a real danger to human health because it leads to several therapeutic resistances to essential drugs, increased morbidity and mortality [9]. The problem of drug falsi-

* Corresponding author at: Laboratory of Analytical Pharmaceutical Chemistry, CIRM, ViBra-Sante Hub, Department of Pharmacy, University of Liege, Avenue Hippocrate 15, B-4000 Liege, Belgium.
E-mail address: pysacre@uliege.be (P-Y. Sacre).

https://doi.org/10.1016/j.talo.2020.100025
Received 9 November 2020; Received in revised form 15 December 2020; Accepted 15 December 2020

fication varies from one continent to another but represents an estimate of more than 10% of the world drug market. In sub-Saharan countries, it is estimated that more than 30% of marketed medicines are of inferior quality; most of them being substandard or degraded medicines. Even if all pharmacological classes are affected, antibiotics and antimalarials remain the most reported [10]. To reduce the scale of this phenomenon, a strengthening of the pharmaceutical inspections and the development of easy-to-use technologies are needed at the national and international levels. Ideed, pharmaceutical industries and agencies in low- and middle-income countries have limited resources, which induce a lack of convergence towards new modern technologies and stringent regulation [11–13].

Since the last decades, vibrational spectroscopic techniques have known a significant gain of interest in this field. For example, near infrared (NIR) and Raman spectroscopy are used to monitor real-time manufacturing processes, but are also increasingly used to authenticate commercially available batches of drugs and to detect substandard and falsified drugs [8,14,15]. These techniques were compared in terms of cost, simplicity and performance [14–16]. Both of them offer major advantages because they provide information on the chemical and physical nature of the sample in a fast, non-destructive and non-invasive way [14,17–20]. It has to be noticed that NIR spectroscopy is now a well-established technique in the pharmaceutical industry for quality control during the manufacturing process and for monitoring drugs on the market [21–23]. Recently, new low-cost handheld technologies have been developed allowing direct applicability on the field [14,15,23–27].

Several measurement modes can be used depending on the sample form: reflection, transmission or transflection [3]. It is important to emphasize that the development of NIR methods for the quantification of solid dosage forms (powders and tablets) presents major advantages by the fact that they allow measurements to be made with little treatment or without prior treatment of the samples [20,28–30]. Despite these advantages, these methods present several challenges due to the importance of the physical parameters on the spectra. [21,25,31]. In addition, the presence of different excipients or different pharmaceutical forms generally force the analyst to develop specific predictive models per formulation. In order to handle this issue, this study investigates the opportunity to analyze samples in solution. This should allow the building of a single regression model making the routine use and maintenance of such models more straightforward. This dilution of samples may be performed easily on the field using pre-filled tubes with the correct amount of dilution medium. This ensures that low-skilled personal is able to perform the tests with the sample preparation reduced to a minimum.

Quinine-based formulations are among the most falsified pharmaceutical products. In recent yers, more than 15% of antimalarials marketed in low- and middle-income countries have been of poor quality, in which quinoline derivatives and quinine account for 21% [32,33]. Recently, several cases of WHO “drug alerts” mentioning the presence of substandard and / or falsified drugs based on quinine salts on the sub-Saharan market have been reported in different countries among which the Democratic Republic of the Congo (DRC) is one of the most affected [34,35]. The falsification of these products represents a major risk to public health since they are used for severe and / or cerebral malaria, as well as for the treatment of *falciparum*-resistant malaria.

The aim of the study was to develop a quantitative method based on NIR spectroscopy to assess the quality of different quinine-based formulations. For that purpose, the methodology is based on a previous study which quantified quinine in oral drops using a benchtop spectrophotometer [36]. This early work is extended to the analysis of solid samples and injectable solutions while using a single protocol with both benchtop and handheld spectrophotometers.

2. Material and methods

2.1. Material

The antimalarial drugs for which the methods were developed included solid and liquid dosage forms (tablets, oral drops and intravenous solutions) of quinine. These three categories of dosage forms represent the majority of the quinine formulations produced or marketed in DRC.

- Tablets contain high dose of quinine sulfate (79 to 84% w/w depending of the nominal content of API of 250 mg, 300 mg and 500 mg). Starch, magnesium stearate, talc, cellulose microcrystalline and colloidal silicon dioxide are the main excipients used in most of the local productions of quinine sulfate. A mixture of these excipients was provided as free samples by Pharmakina Ltd agro-industrial and pharmaceutical company (Bukavu, D.R. Congo).
- Oral drops are composed of quinine di-hydrochloride (20% w/v) and about 1% w/v of excipient (methyl paraben, propyl paraben, ethanol and green chlorophyllin) in aqueous solution.
- Intravenous solutions contain quinine di-hydrochloride (25% w/v) in aqueous solution.

Quinine sulfate and Quinine hydrochloride standards were purchased from Fagron (Belgium). Hydrochloric acid 37% of analytical grade was purchased from VWR (Belgium).

In addition, three previously confirmed falsified samples of Pharmakina quinine sulfate tablets were analyzed. Sample F1 was presented as Pharmakina tablets of 300 mg (without label). Sample F2 was labeled as Pharmakina 500 mg quinine sulfate tablets (batch SD16-L8003, expiry date 08–2021). Sample F3 was labeled as Pharmakina 500 mg quinine sulfate tablets (batch unreadable, expiry date 03–2012). Based on previous handheld Raman analyses, samples F1 and F2 contain metronidazole and sample F3 contains paracetamol instead of quinine sulfate.

2.2. NIR instrumentations

Two kind of NIR spectrophotometers were used during this study: a benchtop Fourier-transform and a dispersive handheld spectrophotometer. Their characteristics are summarized in Table 1. NIR measurements were performed in transmission mode.

- NIR-A: Multipurpose Fourier-transform near infrared spectrophotometer (MPA, Bruker Optics, Ettlingen, Germany) equipped with TE-InGaAs detector. The spectra were collected with the internal transmission module and recorded with Opus 6.5 software (Optics Bruker). Each spectrum corresponds to the average of 32 scans with a resolution of 8 cm $^{-1}$ in the range of 12,800 – 4000 cm^{-1}. A new background was measured before each validation series. Solutions and test sample solutions were directly scanned in 1 mL shell type glass vials.
- NIR-B: Handheld dispersive NIR spectrometer (NIR-M-T1, Innospectra Corp.). Each spectrum corresponds to the average of 32 scans and with a digital resolution of 228, PGA gain of 16 in the range of 11 111 cm^{-1} to 5882 cm^{-1}. The lamp was turned ON for 1 hour before starting the analysis to reach a stable detector’s temperature. Then the background was measured and the lamp remained lit up during the analysis of the whole series. The device was shut down, cooled to room temperature between each validation series. Solutions were directly scanned in Hellma UV quartz cell, 2 mm light path, with a 700 µL filling.

For reflection analysis of powder raw materials, the following equipment were used: NIR-A with the integrating sphere module equipped with an ambient temperature depletion sulfide detector (RT-PbS); NIR-B was replaced by the NIR-S-G1 reflection module (Innospectra Corp.) using the same acquisition parameters.

Table 1
Characteristics of NIR spectrophotometers.

Code name	Vibrational spectroscopy	Manufacturer	Model	Spectral range analyzed (cm^{-1})	Weight (kg)	Price (k€)
NIR A	FT-NIR	Bruker Optics	MPA (solid probe)	12,500 - 4000	benchtop	~ 100
NIR B	dispersive NIR	Innospectra Corp.	NIR-M-T1	11,111 - 5882	0.14	~ 2

Table 2
Parameters and figures of merit of the DD-SIMCA models.

Metrics	DD-SIMCA Models NIR-A	NIR-B
Spectral range	6206–5705 cm^{-1}	1600–1652 nm
Preprocessing	SG(1,2,15) + SNV + MC	SG(1,2,7) + SNV + MC
# PC	3	3
α	0.01	0.01
Sn (VAL)%	100	86.7
Sp (Quinidine)%	100	93.3
Sp (Cinchonine)%	100	100
Sp (Placebo)%	100	100

SG: Savitzky–Golay (derivative, polynomial order, window size)
MC: Mean center
SNV: Standard normal variate
Sn: Sensitivity
Sp: Specificity.

2.3. Preparation samples

2.3.1. Calibration and validation standards

Calibration and validation standards samples were prepared by dissolving reference quinine sulfate and quinine hydrochloride in 1 M HCl solution. Quinine sulfate standards were prepared in the presence of an adequate amount of mixture excipients to mimic tablets using gravimetric data as reference. Four independent series of samples were realized for both calibration and validation with five concentration levels (40, 60, 80, 100 and 125 mg/mL of equivalent quinine base) in which the target concentration for samples was 100 mg/mL.

Before being analyzed, the quinine sulfate standard solutions containing non-soluble excipients were hand shaken for a maximum of 5 min, then left to stand for 10 min to decant. The supernatant was finally filtered through a 4.5 μm filter.

All calibration and validation solutions were prepared in triplicates for each concentration level and were measured three times. For the validation computations, the three predictions were averaged per sample.

For the validation of the qualitative models, solutions of quinidine and cinchonine were prepared at the same five concentrations. In addition, a placebo solution was prepared with the excipients mixture at the target level.

2.3.2. Preparation of test samples

The test solutions were prepared by simple dissolution or dilution of the unit dosage form with the 1 M HCl aqueous solution to make a final solution of 10% w/v of the declared content. The obtained suspensions of tablet test sample were then hand shaken for a maximum of 5 min, then left to stand for 10 min before passing through a 4.5 μm filter as for the quinine sulfate standards.

2.4. Data acquisition and chemometric data preprocessing

Computations and chemometric analyses were carried out in MATLAB (R2018a) (The Mathworks, Inc., Natick, MA, USA). For PLS models, the PLS toolbox v.4.11 (Eigenvector Research, Inc.,Wenatchee, WA, USA) was used.

In order to reduce variability and enhance chemical spectral features, the raw NIR spectra were preprocessed as described in Table 2.

2.5. Chemometric data analysis

2.5.1. DD-SIMCA models

Because of the nature of NIR spectra (large bands heavily overlapped), it is recommended to use a qualitative model prior to the PLS regression model.

In this study, the data-driven SIMCA (DD-SIMCA) was used to build qualitative identification models. DD-SIMCA is based on the building of principal component analysis (PCA) model of the target class, which in our case corresponds to the calibration spectra. Hence, score distance (SD) and orthogonal distance (OD) can be calculated for each future spectrum allowing to determine, at a given confidence level, the acceptance zone for identification [37]. In our case, DD-SIMCA models were also used to discriminate quinine from two structurally related alkaloids namely cinchonine and quinidine. A placebo solution was also projected onto the DD-SIMCA model to ensure that excipients do not interfere with the qualitative models.

The sensitivity was evaluated from the new set (quinine validation set) on DD-SIMCA models, using the following formula:

$$Sensitivity\ (\%) = \frac{True\ Positives}{Positives} * 100 \quad (1)$$

The specificity was evaluated using objects from quinidine, cinchonine and placebo samples on DD-SIMCA models, using the following formula:

$$Specificity\ (\%) = \frac{True\ Negatives}{Negatives} * 100 \quad (2)$$

2.5.2. PLS analysis

Several PLS models were built using different preprocessing methods, combinations of them and considering different number of latent variables.

The selection of appropriate number of latent variables enable avoiding model under or over fitting. A pre-selection of spectral ranges, preprocessing and number of latent variables was performed using the PLS Toolbox model optimizer using RMSEP as quality criterion. The few last selected models were compared based on the accuracy profiles reflecting the routine use of the method [38].

2.5.3. Validation

The NIR predictive models were validated using the total error approach [39] with the acceptance limits of ± 10% and a 5% risk level.

All validation calculations were carried out with E-noval 4.0b (Pharmalex Belgium, Mont-saint-Guibert, Belgium).

2.5.4. Robustness testing of the model

It is important to ensure the robustness of the method for its future use in routine analyses. As some factors can critically affect NIR measurements and thus, the quality of model, it was important to assess the influence of slight changes in parameters related to sample preparation and instrument configuration. Two robustness tests were conducted.

The first one consisted in preparing the validation standards with changes in the acid solution preparation (± 20% from the optimal concentration) in order to evaluate if the HCl concentration affected the performance of the model. Two concentrations (0.8 M HCl and 1.2 M HCl) were tested as new validation series.

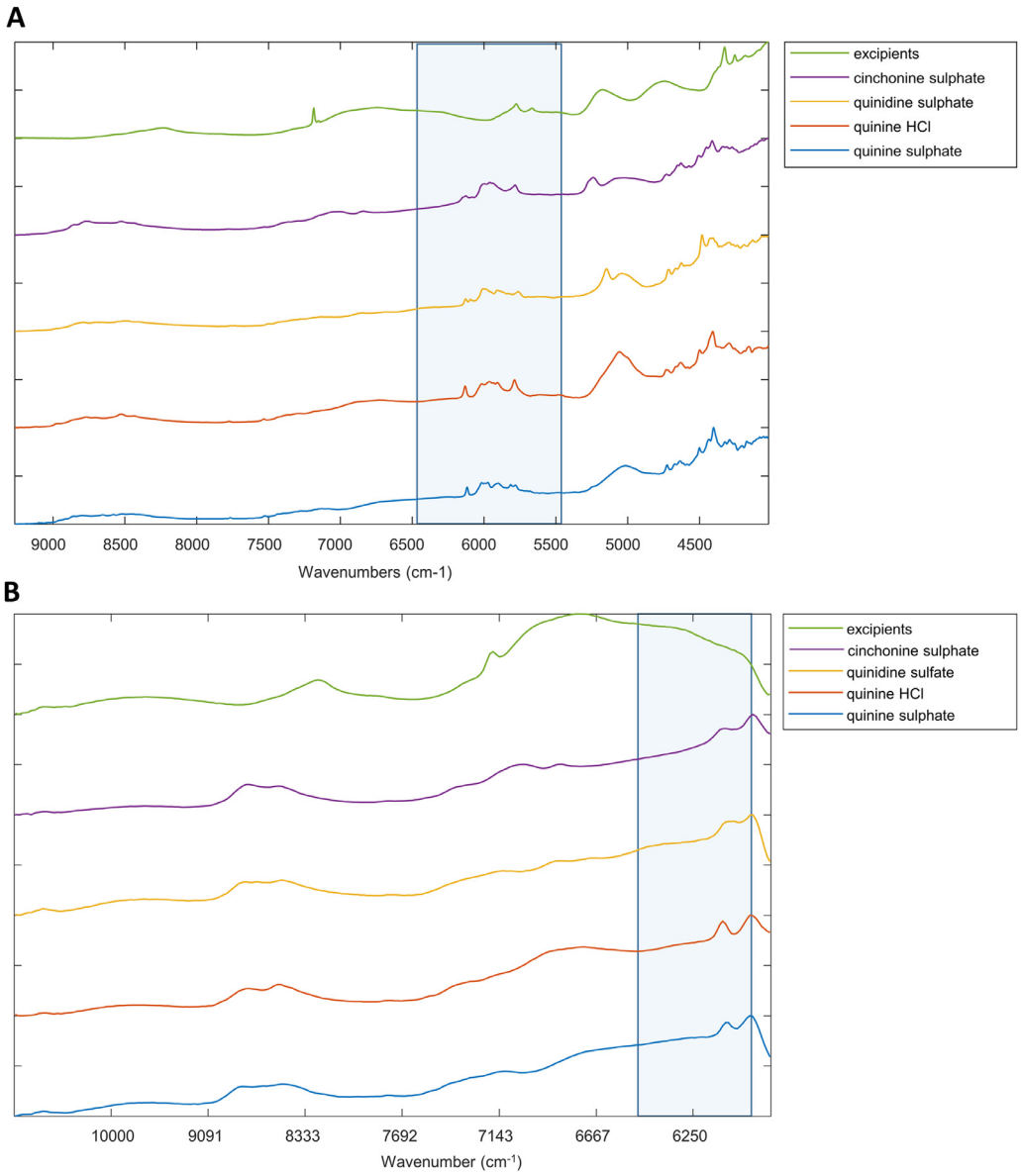

Fig. 1. A. NIR reflection spectra of pure raw materials measured with the NIR-A spectrophotometer. The spectra were offset for better visualization. The accessible spectral range of sample solutions is highlighted in the blue shaded area. B. NIR reflection spectra of pure raw materials measured with the NIR-B spectrophotometer. The spectra were offset for better visualization. The accessible spectral range of sample solutions is highlighted in the blue shaded area. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The second robustness test consisted in maintaining the 1 M HCl solution while modifying acquisition parameters such as the number of scans (16 and 48) and the number of PGA gain (8 and 32). The PGA (Programmable Gain Amplifier) implements a non-inverting amplifier based on an operational amplifier with user programmable gain. The gain can be selected using the configuration window and that between the following values: 1 (0 dB) and 50 (+ 34 dB). Before performing a reference signal and sample signal scan, the PGA gain has been fixed to 16. This will ensure that both sample and reference signals are measured with same PGA gain.

2.5.5. Analysis of routine samples

Samples coming from the local DRC market were analyzed. Three different dosage forms including tablets (250 mg, 300 mg and 500 mg), 20% oral drops and 25% intravenous solutions were tested. Each sample was measured in triplicate.

3. Results and discussion

3.1. Spectral preprocessing

The reflection raw materials spectra (solid state) were recorded with both NIR-A and NIR-B devices and are represented in Fig 1A and 1B respectively. Because the acquisition was conducted on powders, water bands are missing on spectra. It has to be noticed that the extended spectral range of NIR-A is due to the change in detector compared to transmission measurements.

For comparison, spectra recorded with both NIR-A and NIR-B devices in aqueous form are presented in Figure S1. The same spectral region is highlighted in both Fig. 1 and Figure S1 for comparison.

As one can see in Fig. 1, specific spectral features of quinine salts are present in the spectral range where the absorption of O–H bonds of water is weak (blue shaded area). Almost no difference may be observed by

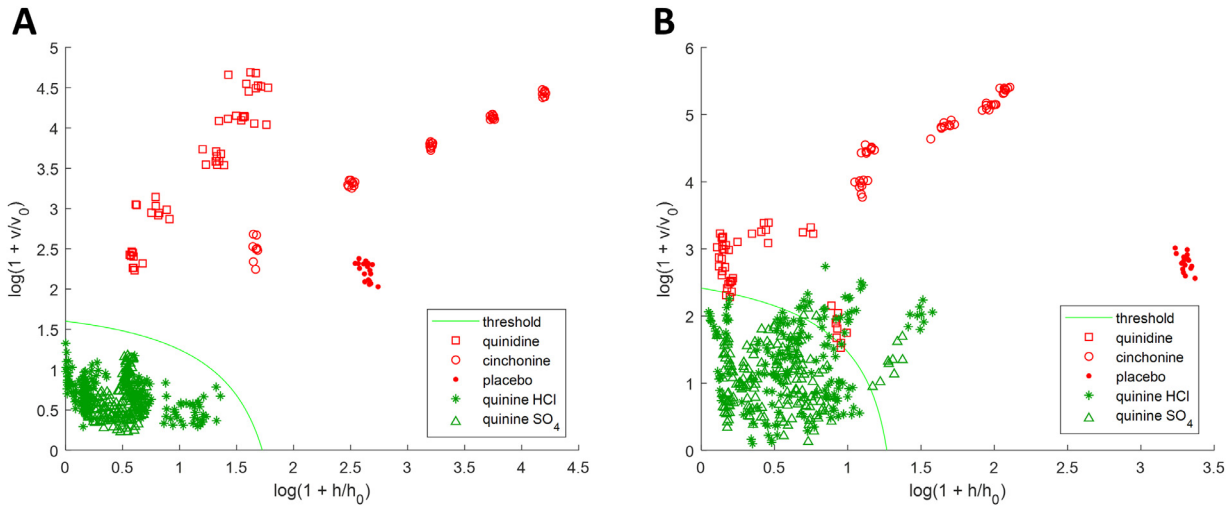

Fig. 2. A. DD-SIMCA acceptance plot for the NIR-A validation samples. Samples below the threshold (green color) are considered as belonging to the target class. The samples above the threshold (red color) are considered as outliers. B. DD-SIMCA acceptance plot for the NIR-B validation samples. Samples below the threshold (green color) are considered as belonging to the target class. The samples above the threshold (red color) are considered as outliers. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

naked eye on the solution spectra. However, chemometrics techniques may detect very small differences in absorption.

3.2. Validation of the quantitative methods

The quantitative models were validated according to the ICH Q2 (R1) guidelines [40] and according to both EMA and FDA guidance for the development and validation of NIR analytical methods [41,42].

3.2.1. Specificity/selectivity

As a first validation criterion, the method specificity should be demonstrated. A good practice is generally to build a qualitative model prior to the quantitative one. This ensures that only spectra similar to the ones that were used for the calibration will be projected onto the regression model.

As it can be observed, visual spectral correlation cannot be a reliable method of quinine selectivity compared to its derivatives such as quinidine and cinchonine. Due to the very weak differences between spectra, preprocessing methods used throughout the manuscript include Savitzky-Golay derivatives to enhance the spectral features and ease the discrimination of the different APIs in the SIMCA models. In addition, chemometric methods were used to help detecting spectral dissimilarities between these three alkaloids. One-class classification models were developed for the two NIR instruments using DD-SIMCA approach. Several preprocessing and spectral ranges were tested for each device. The selected model parameters are listed in Table 2 together with the related sensitivity and specificity. DD-SIMCA acceptance plots for the two NIR spectrometers (NIR-A and NIR-B) are presented in Fig. 2, while Fig. 3 shows the pre-processed NIR spectra acquired in solution using NIR-A and NIR-B. DD-SIMCA models applied to NIR-A data allowed perfect recognition of quinine samples and perfect discrimination of placebo, cinchonine and quinidine samples. This confirms its applicability to reject systematically samples which are not quinine ones prior to quantitative analysis. The performances of the NIR-B device are somewhat less good but this was expected because of the lower resolution and the shorter spectral range. Nevertheless, the performances shown a sensitivity of 87% for quinine samples and a specificity of 93% for quinidine.

These results demonstrate that for the identification of specific quinine-based products the DD-SIMCA models are suitable for both NIR systems.

Table 3
Parameters of the PLS regression models.

Metrics	PLS Models NIR-A	NIR-B
Spectral range	6287–5724 cm^{-1}	6265–5952 cm^{-1}
Preprocessing	SG(1,2,15) + SNV + MC	SG(2,2,9) + MC
# LV	4	5

SG: Savitzky-Golay (derivative, polynomial order, window size)
MC: Mean center
SNV: Standard normal variate.

3.2.2. NIR PLS models

Following the development of the qualitative models, regression models were constructed using the PLS algorithm. The best preprocessing and spectral range are presented together with the optimal number of latent variables (LV) in Table 3.

The obtained results are summarized in Table 4 and the accuracy profiles are presented in Fig. 4. The developed method was successfully validated using the total error approach with acceptance limits of ± 10% and 5.0% for the risk level. The opportunity to use gravimetric data as reference allowed obtaining the best quantitative performances possible since the accumulation of error from the reference method is avoided.

The weights on the first latent variable (LV, representing 99.51% of the covariance captured by the model) were compared to the preprocessed reflection spectra of quinine sulfate and quinine HCl for the NIR-A device (See Fig. 5A). The same was done for NIR-B comparing the preprocessed reflection spectra of quinine sulfate and quinine HCl to the weights on the first and the second latent variables (representing 44.13 and 55.47% of the covariance captured by the model, respectively) (See Fig. 5B). As one can see, the weights on the latent variables for NIR A and B are, on the one hand, in accordance with the preprocessed spectra of quinine HCl. On the other hand, quinine sulfate spectra are different from the weights on the LVs. This is due to that the loading weights origin from NIR spectra was recorded in the liquid phase, while the pre-processed NIR spectra were recorded on the solid phase, which explained the presence of chloride ions in the HCl solution that will replace all the sulfate ions. Therefore, all quinine present in the solution will have the same NIR spectrum facilitating the development of a single regression model for the analysis of both quinine HCl and quinine

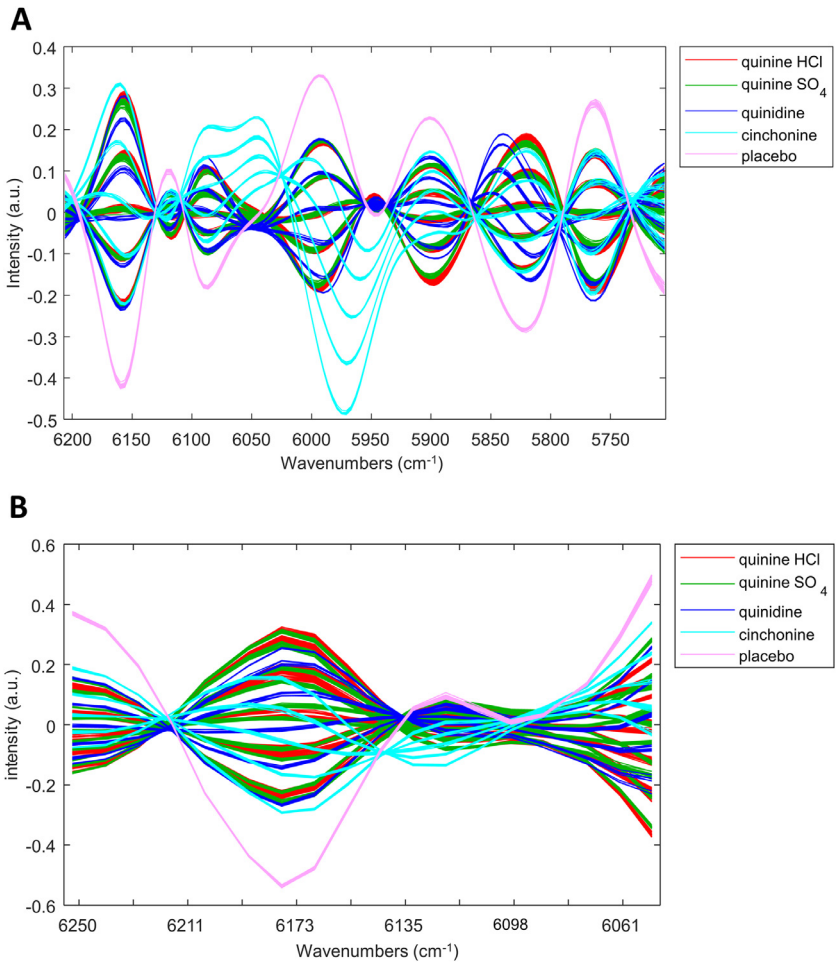

Fig. 3. A. NIR preprocessed spectra acquired with the NIR-A spectrophotometer. The spectral range and the preprocessing are the ones used for the DD-SIMCA model. For each API (except placebo), the five concentration level solutions are presented. B. NIR preprocessed spectra acquired with the NIR-B spectrophotometer. The spectral range and the preprocessing are the ones used for the DD-SIMCA model. For each API (except placebo), the five concentration level solutions are presented.

Table 4
ICH Q2 (R1) validation criteria values of the PLS regression models.

	Concentration level (mg/mL of equivalent quinine base)	NIR-A	NIR-B
Trueness Relative bias (%)	41.05	0.635	−0.109
	61.55	0.297	0.801
	82.06	0.247	0.476
	102.6	0.307	0.109
	123.1	0.097	−0.463
Intra-assay precision Repeatability (RSD%)	41.05	0.857	1.654
	61.55	0.563	1.248
	82.06	0.730	0.931
	102.6	0.777	1.101
	123.1	0.800	1.555
Between-assay precision Intermediate precision (RSD%)	41.05	0.976	1.654
	61.55	0.611	1.248
	82.06	0.730	0.931
	102.6	0.836	1.118
	123.1	0.800	1.555
Accuracy Relative β -expectation tolerance limits (%)	41.05	[−1.53, 2.80]	[−3.60, 3.39]
	61.55	[−1.03, 1.62]	[−1.84, 3.44]
	82.06	[−1.29, 1.79]	[−1.49, 2.44]
	102.6	[−1.50, 2.11]	[−2.26, 2.48]
	123.1	[−1.59, 1.78]	[−3.75, 2.82]

sulfate samples. In addition, a slight shift in NIR absorbance is observed between powder and solution spectra. This is due to the change of polarity of the medium that will induce a slight change in the polarization of the molecular bonds.

This comparison demonstrates also the specificity of the regression model (as suggested by [41,42]) since more than 99% of the covariance captured by the model is highly correlated to the NIR spectrum of quinine.

3.2.3. *Robustness test results*

Deliberate variations in the operating conditions such as the concentration of the acid solution were evaluated for their influence on the

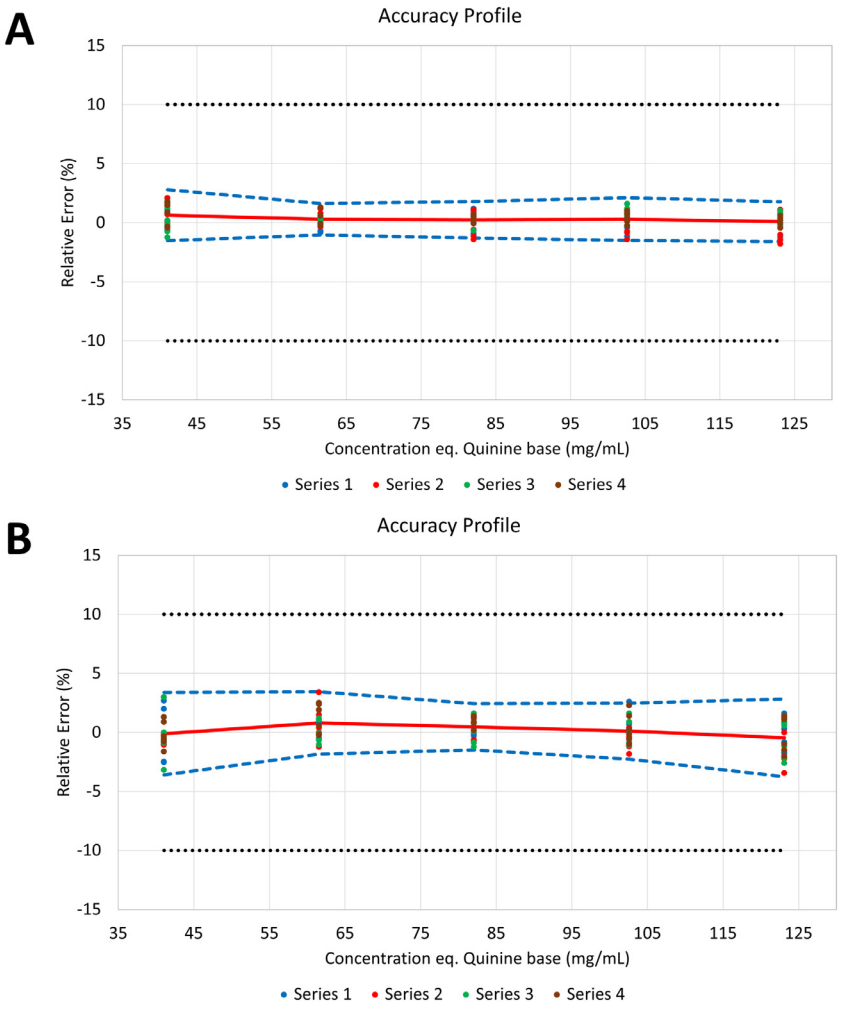

Fig. 4. A. Accuracy profile obtained for the four validation series with the NIR-A spectrophotometer using an acceptance limit of +/- 10% and a risk level set at 5%. The plain red line is the relative bias, the dashed blue lines are the β-expectation tolerance limits, and the dashed black lines represent the acceptance limits. The dots represent the relative error of the results and are plotted with respect to their targeted concentration. B. Accuracy profile obtained for the four validation series with the NIR-B spectrophotometer using an acceptance limit of +/- 10% and a risk level set at 5%. The plain red line is the relative bias, the dashed blue lines are the β-expectation tolerance limits, and the dashed black lines represent the acceptance limits. The dots represent the relative error of the results and are plotted with respect to their targeted concentration. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

accuracy profile. For that purpose, two additional validation series (series 5 with HCl 0.8 M and series 6 with HCl 1.2 M) were measured and integrated in the validation series to check whether a slight change in the concentration of the dissolution medium could have an effect on the accuracy profile. The results obtained showed that the concentration factor of the dissolution medium has no significant effect on the predictive performance of the models since their accuracy profile are still within the acceptance limits (see Figures S2 and S3).

However, when looking closer to the prediction results, one can see that the 0.8 M solution (series 5) results in a slight positive bias for both NIR-A and NIR-B. On the contrary, the 1.2 M solution (series 6) results in a negative bias for NIR-A and a comparable bias to the 1 M solution for NIR-B (see Figures S4 and S5). The relative repeatability and intermediate precision were slightly increased but the total error remained low with the beta-expectation tolerance intervals completely comprised within the acceptance limits.

This showed the robustness of the method to a modification of the concentration of the acid solution; which would guarantee a predictive performance of the two models in the routine analyzes.

The second robustness test consisted in evaluating the influence of deliberate variations of the number of PGA gains or the number of scans on the accuracy profile. The option here was to maintain constant one parameter at the optimal value while varying the other by two values around the optimal value. Four series were prepared with HCl 1 M and tested to evaluate the effect of a slight change in the NIR-B acquisition parameter on the accuracy profile. The results obtained showed that the number of PGA gain and number of scans factors have no significant effect on the predictive performance of the models since their accuracy profile are still within the acceptance limits (data not shown).

3.3. Analysis of samples

The validated models were used to analyze 26 samples of different dosage forms collected in the local DR.. Congo pharmaceutical market. The samples were of three dosage forms (tablets, oral drops and intravenous solutions), with different auxiliary ingredients or excipients. In addition, three falsified “Pharmakina” quinine sulfate tablets were also analyzed.

Before being quantified by the PLS model, sample solutions were projected onto the DD-SIMCA models confirming their identity (see Figs. 6 and 7). As expected, original Pharmakina products were accepted by the DD-SIMCA models whereas falsified products were rejected.

Each sample solution was analyzed in triplicate. The average results per sample are presented in Table 5. All samples have been found to be within the acceptance limits of ±10.0% of the declared value except samples number 25 and 26 which were in the upper limit of specification and/or out of specification. These results suggest an overdose of that batch as these two samples are of the same manufacturing batch. These results show that the developed models could quantitatively be used to analyze most of quinine formulations. The advantage of dissolving quinine is the possibility to analyze with the same PLS model both tablets, oral drops and intravenous solutions.

NIR-B has shown similar analytical performance to NIR-A in quantifying quinine in different formulations without using the same spectral

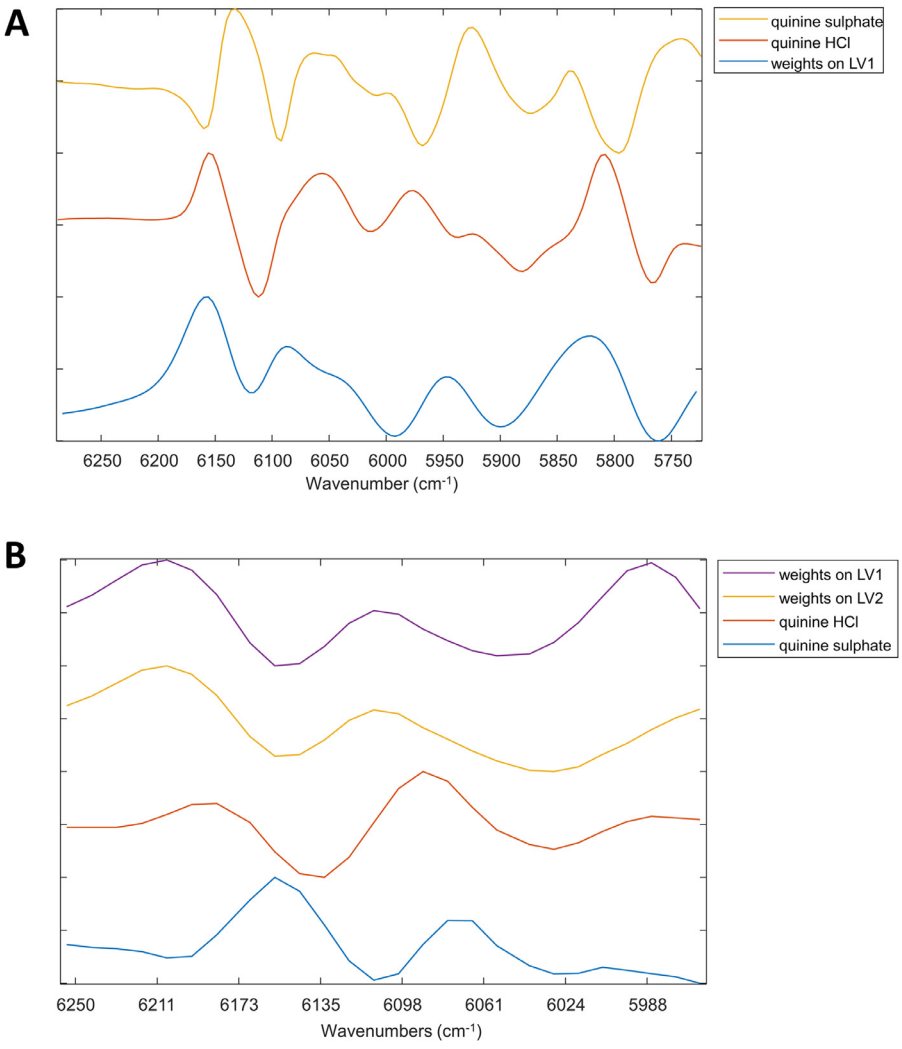

Fig. 5. A. Weights on the first latent variable (LV1) presented with the reflection spectra of pure quinine sulfate and quinine HCl measured with the NIR-A spectrophotometer. The spectra are offset for better visualization. The spectral range and the preprocessing are the ones of the PLS regression model. B. Weights on the first two latent variables (LV1 and LV2) presented with the reflection spectra of pure quinine sulfate and quinine HCl measured with the NIR-B spectrophotometer. The spectra are offset for better visualization. The spectral range and the preprocessing are the ones of the PLS regression model.

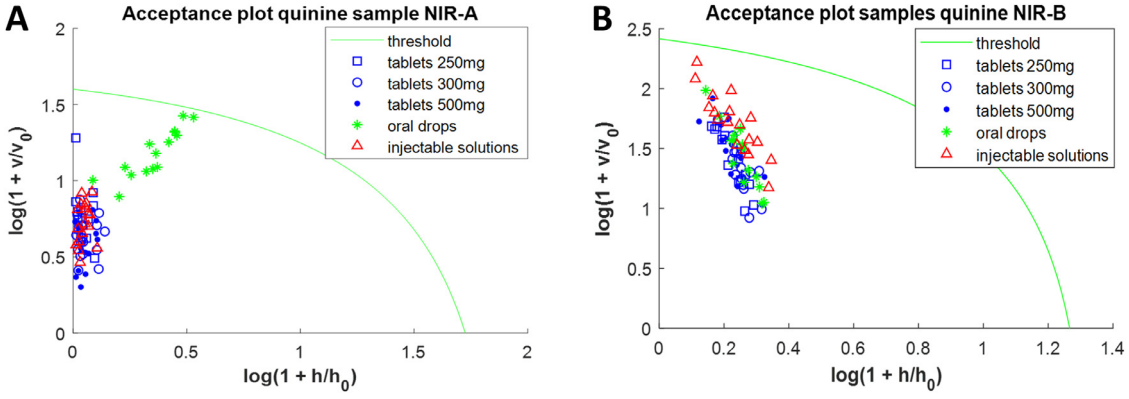

Fig. 6. A. DD-SIMCA acceptance plot of the quinine samples analyzed with the NIR-A spectrophotometer. Samples are considered as belonging to the target class if they are below the threshold in the left corner of the plot. B. DD-SIMCA acceptance plot of the quinine samples analyzed with the NIR-B spectrophotometer. Samples are considered as belonging to the target class if they are below the threshold in the left corner of the plot.

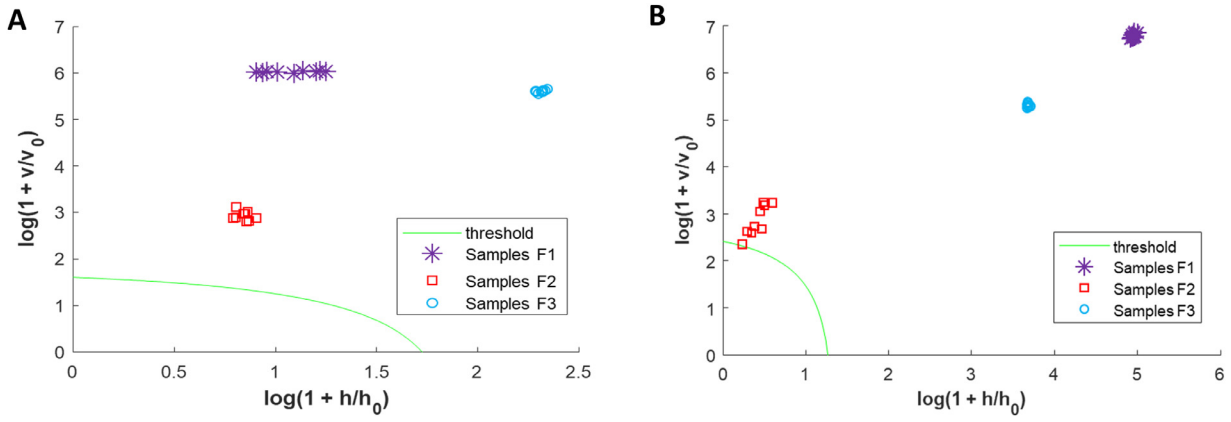

Fig. 7. A. DD-SIMCA acceptance plot of the falsified quinine samples analyzed with the NIR-A spectrophotometer. Samples are considered as belonging to the target class if they are below the threshold in the left corner of the plot. B. DD-SIMCA acceptance plot of the falsified quinine samples analyzed with the NIR-B spectrophotometer. Samples are considered as belonging to the target class if they are below the threshold in the left corner of the plot.

Table 5
Assay results for quinine samples collected in the local DR.. Congo market for the NIR-A and the NIR-B spectrophotometer.

Sample	Dosage form	Manufacture	Country of origin	Batch N°	NIR-A % of claimed value	NIR-B % of claimed value
1	Drops 20%	Pharmakina	Bukavu/DRC	B60/19	100.5	103.3
2	Drops 20%	Pharmakina	Bukavu/DRC	B21/19	100.9	101.6
3	Drops 20%	Pharmakina	Bukavu/DRC	B31/19	100.2	101.4
4	Drops 20%	Pharmakina	Bukavu/DRC	B37/19	100.4	100.5
5	Drops 20%	Pharmakina	Bukavu/DRC	B56/ 19	99.4	101.4
6	Tablet 250 mg	Pharmakina	Bukavu/DRC	SC20–0014	91.7	91.4
7	Tablet 250 mg	Pharmakina	Bukavu/DRC	SC20–0014	94.9	93.9
8	Tablet 250 mg	Pharmakina	Bukavu/DRC	SC20–0014	93.9	91.7
9	Tablet 250 mg	Pharmakina	Bukavu/DRC	SC20–0014	94.7	93.6
10	Tablet 250 mg	Pharmakina	Bukavu/DRC	SC20–0014	93.8	92.6
11	Tablet 300 mg	Pharmakina	Bukavu/DRC	SB17–0068B	97.5	96.7
12	Tablet 300 mg	Pharmakina	Bukavu/DRC	SB17–0068B	95.6	94.5
13	Tablet 300 mg	Pharmakina	Bukavu/DRC	SB17–0068B	97.1	96.1
14	Tablet 300 mg	Pharmakina	Bukavu/DRC	SB17–0068B	96.9	95.4
15	Tablet 300 mg	Pharmakina	Bukavu/DRC	SB17–0068B	96.5	95.8
16	Tablet 500 mg	Pharmakina	Bukavu/DRC	SD17–0097C	97.5	96.8
17	Tablet 500 mg	Pharmakina	Bukavu/DRC	SD17–0097C	100.6	99.2
18	Tablet 500 mg	Pharmakina	Bukavu/DRC	SD17–0097C	99.2	97.5
19	Tablet 500 mg	Pharmakina	Bukavu/DRC	SD17–0097C	98.9	98.1
20	Tablet 500 mg	Pharmakina	Bukavu/DRC	SD17–0097C	99.0	97.6
21	Injectables 500 mg/2mL	New Cesamex	Kinshasa/DRC	190,127	107.3	108.5
22	Injectables 500 mg/2mL	New Cesamex	Kinshasa/DRC	190,127	106.2	106.3
23	Injectables 500 mg/2mL	Shangai Ruilli IMP C.O.LTD	China	190,728	106.0	104.1
24	Injectables 500 mg/2mL	Shangai Ruilli IMP C.O.LTD	China	190,728	106.2	104.3
25	Injectables 500 mg/2mL	Chengshi Manufacture Mediane C.O. LTD	China	190,466	110.0	109.1
26	Injectables 500 mg/2mL	Chengshi Manufacture Mediane C.O. LTD	China	190,466	111.8	112.9

region. In addition, NIR-B has good robustness in terms of predictive performance, which can encourage its use in the field. Further studies are ongoing to evaluate the environmental influence such as the temperature and relative humidity for its application in tropical areas.

4. Conclusion

Handheld spectrophotometers have now become staple tools for the quality control of pharmaceuticals and more particularly for the field detection of substandard and falsified medicines. In this frame, a practical, a low-cost and easy to implement method for the qualitative and quantitative analysis of quinine-based samples is proposed.

The proposed method requires little sample preparation consisting of its dissolution in a HCl 1 M dilution medium. The choice of this medium was justified because it is inexpensive and easily reachable in low and middle-income countries. The opportunity to dissolve solid samples in HCl allows the construction and maintenance of a single regression model for different formulations including liquid samples with different quinine salts (sulfate or di-hydrochloride).

The method was fully validated using the total error approach within ± 10% as acceptance limits and with a risk of 5%. The methods also passed the robustness tests in terms of the factors related to sample preparation. A comparison of developed methods showed that both could be used to verify the quality of quinine samples. Twenty-six samples collected in the Congolese pharmaceutical market were analyzed by both NIR instruments. Certain limits could nevertheless be found concerning the use on ground in tropical zones of Africa where one often observes high and various relative humidity and high temperatures knowing that these environmental factors could affect the performance of the analyzes. Another limitation is the analysis of quinine syrup. Indeed, the very high amount of sugar makes it impossible to analyze this kind of sample with the proposed method.

This study constitutes the first step for the implementation of a field quinine quality control method based on NIR techniques based on transmission mode able to detect both falsifications but also sub-

standard medicines. The opportunity to work in transmission mode has several advantages over reflectance mode. First, it allows the analysis of the whole tablet. It is now commonly admitted that transmission spectroscopy is much more representative of the sample compared to reflectance that analyzes only the surface. Second, the advantage of working in solution is that several pharmaceutical forms may be analyzed with the same regression model. This facilitates the maintenance of the model and its implementation compared to the need of the construction and validation of a specific regression model for each specialty in reflectance mode.

Declaration of Competing Interest

We declare no conflict of interest regarding the publication entitled "Application of NIR handheld transmission spectroscopy and chemometrics to assess the quality of locally produced antimalarial medicines in the Democratic Republic of Congo."

Acknowledgments

This work was done with the financial support of the Academy of Research and Higher Education is the federation of French-speaking higher education institutions in Belgium (ARES). We thank the coordination team of PFS-2016 project and PRD-2018 project. The financial support of this research by the Walloon Region of Belgium in the framework of the Vibra4Fake project (convention n°:7517) is gratefully acknowledged.

The European funds of regional development (FEDER – PHARE project) and the Walloon Region program "Walloon-2020.EU" are also thanked for the funding of L. Coïc

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.talo.2020.100025.

References

[1] K.A. Bakeev, Process Analytical Technology, Wiley, Blackwell Publishing Ltd, Oxford, UK, 2010, doi:10.1002/9780470689592.
[2] G. Allison, Y.T. Cain, C. Cooney, T. Garcia, T.G. Bizjak, O. Holte, N. Jagota, B. Komas, E. Korakianiti, D. Kourti, R. Madurawe, E. Morefield, F. Montgomery, M. Nasr, W. Randolph, J.-.L. Robert, D. Rudd, D. Zezza, Regulatory and quality considerations for continuous manufacturing May 20–21, 2014 continuous manufacturing symposium, J. Pharm. Sci. 104 (2015) 803–812, doi:10.1002/jps.24324.
[3] F.D.A. US, Guidance For Industry Guidance For Industry PAT — A Framework For Innovative Pharmaceutical, 2004.
[4] F. De Leersnyder, V. Vanhoorne, A. Kumar, C. Vervaet, T. De Beer, Evaluation of an in-line NIR spectroscopic method for the determination of the residence time in a tablet press, Int. J. Pharm. 565 (2019) 358–366, doi:10.1016/j.ijpharm.2019.05.006.
[5] B. Igne, C. Airiau, S. Talwar, E. Towns, Chemometrics in the pharmaceutical industry, in: Compr. Chemom., 2nd Ed., Elsevier, 2020, pp. 33–68, doi:10.1016/B978-0-12-409547-2.14638-4.
[6] B. Nagy, D. Petra, D.L. Galata, B. Démuth, E. Borbás, G. Marosi, Z.K. Nagy, A. Farkas, Application of artificial neural networks for process analytical technology-based dissolution testing, Int. J. Pharm. 567 (2019) 118464, doi:10.1016/J.IJPHARM.2019.118464.
[7] C. Lourenco, N. Orphanos, C. Parker, The international council for harmonisation: positioning for the future with its recent reform and over 25 years of harmonisation work, Pharm. Policy Law. 18 (2016) 79–89, doi:10.3233/PPL-160434.
[8] WHO, Substandard and falsified medical products - Fact Sheet, (2018). http://www.who.int/news-room/fact-sheets/detail/substandard-and-falsified-medical-products.
[9] V. Brower, Falsified and substandard malaria drugs in Africa, Lancet Infect. Dis. 17 (2017) 1026–1027, doi:10.1016/S1473-3099(17)30528-5.
[10] Y. Tie, C. Vanhee, E. Deconinck, E. Adams, Development and validation of chromatographic methods for screening and subsequent quantification of suspected illegal antimicrobial drugs encountered on the Belgian market, Talanta 194 (2019) 876–887, doi:10.1016/j.talanta.2018.10.078.
[11] J. Mayer, Globalization, technology transfer and skill accumulation in low-income countries, in: 2002. doi:10.4324/9780203427637.ch5.
[12] World Health Organization, Local production and access to medicines in low- and middle-income countries: a literature review and critical analysis, 2011.
[13] F. Praussello, Globalization and incomplete technology transfer to developing countries, in: Glob. Third World, Palgrave Macmillan, UK, London, 2006, pp. 200–216, doi:10.1057/9780230502567_12.
[14] P.H. Ciza, P.-Y. Sacre, C. Waffo, L. Coïc, H. Avohou, J.K. Mbinze, R. Ngono, R.D. Marini, P. Hubert, E. Ziemons, Comparing the qualitative performances of handheld NIR and Raman spectrophotometers for the detection of falsified pharmaceutical products, Talanta 202 (2019) 469–478, doi:10.1016/j.talanta.2019.04.049.
[15] R. Deidda, P.-Y. Sacre, M. Clavaud, L. Coïc, H. Avohou, Ph. Hubert, E. Ziemons, Vibrational spectroscopy in analysis of pharmaceuticals: critical review of innovative portable and handheld NIR and Raman spectrophotometers, TrAC Trends Anal. Chem. 114 (2019) 251–259, doi:10.1016/j.trac.2019.02.035.
[16] S. Vickers, M. Bernier, S. Zambrzycki, F.M. Fernandez, P.N. Newton, C. Caillet, Field detection devices for screening the quality of medicines: a systematic review, BMJ Glob. Heal. 3 (2018) e000725, doi:10.1136/bmjgh-2018-000725.
[17] H. Rasheed, L. Höllein, U. Holzgrabe, Future information technology tools for fighting substandard and falsified medicines in low- and middle-income countries, Front. Pharmacol. 9 (2018) 995, doi:10.3389/fphar.2018.00995.
[18] N. Fuenffinger, S. Arzhantsev, C. Gryniewicz-Ruzicka, Classification of ciprofloxacin tablets using near-infrared spectroscopy and chemometric modeling, Appl. Spectrosc. 0 (2017) 3702817699624, doi:10.1177/0003702817699624.
[19] W.-.B. Zou, L.-.H. Yin, S.-.H. Jin, Advances in rapid drug detection technology, J. Pharm. Biomed. Anal. 147 (2018) 81–88, doi:10.1016/j.jpba.2017.08.016.
[20] J.A. Griffen, A.W. Owen, J. Burley, V. Taresco, P. Matousek, Rapid quantification of low level polymorph content in a solid dose form using transmission Raman spectroscopy, J. Pharm. Biomed. Anal. 128 (2016) 35–45, doi:10.1016/j.jpba.2016.05.017.
[21] T. Pedersen, J. Rantanen, K. Naelapää, E. Skibsted, Near infrared analysis of pharmaceutical powders with empirical target distribution optimization (ETDO), J. Pharm. Biomed. Anal. 181 (2020) 113059, doi:10.1016/j.jpba.2019.113059.
[22] Y. Hattori, M. Sugata, H. Kamata, M. Nagata, T. Nagato, K. Hasegawa, M. Otsuka, Real-time monitoring of the tablet-coating process by near-infrared spectroscopy - effects of coating polymer concentrations on pharmaceutical properties of tablets, J. Drug Deliv. Sci. Technol. 46 (2018) 111–121, doi:10.1016/J.JDDST.2018.04.018.
[23] C. Pasquini, Near infrared spectroscopy: a mature analytical technique with new perspectives – a review, Anal. Chim. Acta. 1026 (2018) 8–36, doi:10.1016/j.aca.2018.04.004.
[24] L. Coic, P.-Y. Sacré, A. Dispas, E. Dumont, J. Horne, C. De Bleye, M. Fillet, Ph. Hubert, E. Ziemons, Evaluation of the analytical performances of two Raman handheld spectrophotometers for pharmaceutical solid dosage form quantitation, Talanta 214 (2020) 120888, doi:10.1016/j.talanta.2020.120888.
[25] H. Yan, H.W. Siesler, Quantitative analysis of a pharmaceutical formulation: performance comparison of different handheld near-infrared spectrometers, J. Pharm. Biomed. Anal. 160 (2018) 179–186, doi:10.1016/j.jpba.2018.07.048.
[26] R.B. Chavan, N. Bhargavi, A. Lodagekar, N.R. Shastri, Near infra red spectroscopy: a tool for solid state characterization, Drug Discov. Today. 22 (2017) 1835–1843, doi:10.1016/j.drudis.2017.09.002.
[27] J. Qin, M.S. Kim, K. Chao, S. Dhakal, B.-.K. Cho, S. Lohumi, C. Mo, Y. Peng, M. Huang, Advances in Raman spectroscopy and imaging techniques for quality and safety inspection of horticultural products, Postharvest Biol. Technol. 149 (2019) 101–117, doi:10.1016/j.postharvbio.2018.11.004.
[28] M.C. Hennigan, A.G. Ryder, Quantitative polymorph contaminant analysis in tablets using Raman and near infra-red spectroscopies, J. Pharm. Biomed. Anal. 72 (2013) 163–171, doi:10.1016/j.jpba.2012.10.002.
[29] A. Heinz, M. Savolainen, T. Rades, C.J. Strachan, Quantifying ternary mixtures of different solid-state forms of indomethacin by Raman and near-infrared spectroscopy, Eur. J. Pharm. Sci. 32 (2007) 182–192, doi:10.1016/j.ejps.2007.07.003.
[30] C.M. McGoverin, L.C.H. Ho, J.A. Zeitler, C.J. Strachan, K.C. Gordon, T. Rades, Quantification of binary polymorphic mixtures of ranitidine hydrochloride using NIR spectroscopy, Vib. Spectrosc. 41 (2006) 225–231, doi:10.1016/j.vibspec.2006.04.026.
[31] M. Blanco, A. Peguero, Influence of physical factors on the accuracy of calibration models for NIR spectroscopy, J. Pharm. Biomed. Anal. 52 (2010) 59–65, doi:10.1016/j.jpba.2009.12.009.
[32] J. Grech, J. Robertson, J. Thomas, G. Cooper, M. Naunton, T. Kelly, An empirical review of antimalarial quality field surveys: the importance of characterising outcomes, J. Pharm. Biomed. Anal. 147 (2018) 612–623, doi:10.1016/j.jpba.2017.04.056.
[33] S.M. Beargie, C.R. Higgins, D.R. Evans, S.K. Laing, D. Erim, S. Ozawa, The economic impact of substandard and falsified antimalarial medications in Nigeria, PLoS ONE 14 (2019) e0217910, doi:10.1371/journal.pone.0217910.
[34] WHOMedical Product Alert N° 2/2017, WHO, 2017.
[35] WHOMedical Product Alert N° 10/2019, WHO, 2019.
[36] J.K. Mbinze, P.-.Y. Sacré, A. Yemoa, J. Mavar Tayey Mbay, V. Habyalimana, N. Kalenda, P. Hubert, R.D. Marini, E. Ziemons, Development, validation and comparison of NIR and Raman methods for the identification and assay of poor-quality oral quinine drops, J. Pharm. Biomed. Anal. 111 (2015) 21–27, doi:10.1016/j.jpba.2015.02.049.
[37] Y.V. Zontov, O.Y. Rodionova, S.V. Kucheryavskiy, A.L. Pomerantsev, DD-SIMCA — A MATLAB GUI tool for data driven SIMCA approach, Chemom. Intell. Lab. Syst. 167 (2017) 23–28, doi:10.1016/j.chemolab.2017.05.010.
[38] C. De Bleye, P.F. Chavez, J. Mantanus, R. Marini, P. Hubert, E. Rozet, E. Ziemons, Critical review of near-infrared spectroscopic methods validations in pharmaceutical applications, J. Pharm. Biomed. Anal. 69 (2012) 125–132, doi:10.1016/j.jpba.2012.02.003.
[39] Ph. Hubert, J.-J. Nguyen-Huu, B. Boulanger, E. Chapuzet, P. Chiap, N. Cohen, P.-A. Compagnon, W. Dewé, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier,

G. Muzard, C. Nivet, L. Valat, W. Dewe, M. Feinberg, M. Lallier, M. Laurentie, N. Mercier, G. Muzard, C. Nivet, L. Valat, Harmonization of strategies for the validation of quantitative analytical procedures. A SFSTP proposal–Part I, J. Pharm. Biomed. Anal. 36 (2004) 579–586, doi:10.1016/j.jpba.2004.07.027.

[40] ICH, Validation of a analytical procedures : text and methodology Q2(R1), Guidance 1994 (2005) 17.

[41] Food and Drug Administration, Development and submission of near infrared analytical procedures guidance for industry, (2015).

[42] European Medicines Agency, Guideline on the use of Near Infrared Spectroscopy (NIRS) by the pharmaceutical industry and the data requirements for new submissions and variations, Guideline-EMEA/CHMP/CVMP/QWP/17760/2009 44 (2014) 1–28.