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Comparison of several strategies for the deployment of a multivariate regression model on several handheld NIR instruments. Application to the quality control of medicines

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ABSTRACT

Chemometrics applied to spectroscopic measurements such as near-infrared are gaining more and more importance for quality control of pharmaceutical products. Handheld near-infrared devices show great promise as a medicines quality screening technique for post-marketing surveillance. These devices are able to detect substandard and falsified medicines in pharmaceutical supply chains and enable rapid action before these medicines reach patients. The instrumental and

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environmental changes, expected or not, can adversely affect the analytical performances of prediction models developed for routine applications. Based on a previous study, PLS prediction models were developed and validated on three similar handheld NIR transmission spectrophotometers of the same model and from same company. These models have shown to be effective in analyzing metformin tablet samples, but significant spectral differences between handheld systems complicated their deployment for routine analysis. In this study, different strategies have been applied and compared to correct the instrumental variations, including global modelling (GM) and calibration transfer methods (Direct Standardization, DS; Spectral Space Transformation, SST and Slope/Bias correction, SBC), considering the RMSEP and the accuracy profile as assessment criteria. The transfer methods showed good capabilities to maintain the predictive performances comparable to that of the global modelling approach, except for a remaining slight bias. This approach is interesting since very few standardization samples are required to develop an adequate transfer model. GM, SST and SBC were able to correct/handle drifts in the spectral responses of different handheld instruments and thus may help to avoid the need for a long, laborious, and costly full recalibration process due to inter-instrument variations.

1. Introduction

The development of pharmaceutical products has led to an innovative therapeutic revolution in human health. However, quality is one of the important attributes that a medical product (medicine) must have throughout its life cycle in order to meet the needs for which it was designed. Substandard and falsified (SF) medicines pose a risk to public health. The main cause of substandard drugs is the failure of good manufacturing practices and good distribution practices, while falsification is the result of fraudulent activity for the purpose of economic benefit.

A physicochemical analysis is one of the most important ways to assess the quality medicines and possibly detect SF medicines. Several chemical and instrumental methods have been developed and described in the literature among which the vibrational spectroscopic methods offer several advantages in that they are non-invasive, fast and easy to use [1–4]. Among the vibrational spectroscopic techniques, near-infrared spectroscopy (NIR) is one of the most used since it allows rapid sensing of the physical and chemical properties of a sample. The physicochemical characteristics of a pharmaceutical sample are determined by several factors involved in the manufacturing process such as the nature and composition of the ingredients and the operating

parameters and conditions. Therefore, NIR spectra may be considered fingerprints of a pharmaceutical formulation and may be used to identify falsified or substandard samples [5]. However, NIR spectroscopy has certain limitations in interpreting information from raw spectral data. In this context, chemometric tools are used.

Chemometrics is a discipline of chemical sciences using mathematical and statistical tools and is a mandatory step in the treatment of NIR spectra since it allows the establishment of a relationship between the measured spectra and the physicochemical properties of the sample [6, 7]. Generally, in vibrational spectroscopy, the predictive modelling is based on a set of known samples called “calibration set” or “training set”, which will allow to establish the relationship between the measured spectra and the property of interest. In general, the development of a representative and robust regression model for pharmaceutical products takes a lot of time and is expensive [8].

In the past decade, handheld and portable NIR spectrophotometers have known an increasing interest with the marketing of several low- cost devices [9,10]. However, if the hardware evolution now allows a lot of low-skilled workers to analyze suspicious samples directly in the field, the major bottleneck is situated in the data analysis part. Indeed, because of the high influence of the physical and chemical parameters on the measured NIR spectra, it is almost necessary to build a specific regression model for each pharmaceutical formulation. This represents a huge amount of work and is almost impossible to implement for the mass screening of medicines regarding the diversity of formulations on the market. These limitations hinder the applicability of NIR spectroscopy in the detection of substandard medicines. Therefore, in a previous paper, we proposed a rapid methodology involving a minimal sample preparation that allowed the quantitative analysis of several quinine-based formulations and dosage forms with a single regression model [11]. This approach facilitates the maintenance of the unique model and its implementation in routine analysis.

However, the regression model was developed based on the training data of a given instrument and under stable environmental conditions (temperature and humidity). The predictions could fail if the spectra of the tested samples are measured on a different instrument without any prior standardization and / or under different environmental conditions [12,13]. This could happen if the calibration data of the primary instrument has a significant instrumental or environmental contribution on the developed model [13]. In such case, the adjustment or maintenance of the calibration model, by correcting the instrumental or environmental drifts, remains very important to

ensure its performances in routine analysis. The solution would therefore be to consider different approaches. The first approach is a global modelling which allows inclusion of new sample data from different instrument or under different environmental conditions [14]. This permits to consider the new contributions from instrumental or environmental effects. But this strategy will not be feasible on a large scale if one must measure new calibration sets on each new equipment used. The second approach leads to design the original experiments to avoid contributions effects on the responses. The problem of this approach is that those contribution effects must be known in advance which is usually not possible. The third approach consists of doing some mathematical treatment of the data from the two instruments (primary instrument and secondary instrument). The goal of this approach is to isolate and compensate the spectral differences between primary instrument and secondary instrument [12–19]. This approach seems to be much more practical and adequate in the pharmaceutical field insofar as it would make it possible to guarantee the predictive performance of the model whatever the change in instrumental or environmental conditions. This can be achieved by instrumental standardization methods or calibration transfer methods.

Many calibration transfer approaches have been developed and discussed in the literature [13–15,17,20–30]. For spectroscopic instruments, several calibration transfer methods have been tested for the maintenance of models over time. The standardization methods make it possible to map the space of the signals of the measurement system to the space of the calibration system. Some other methods allow removing variation in responses of different instruments (e.g. orthogonal signal correction, OSC and generalized least squares weighting, GLSW) [31–35]. The spectral space transformation (SST) method allows to eliminate spectral differences induced by instrumental changes. It performs transformation between two spectral spaces spanned by the corresponding spectra of a subset of standardization samples measured on two instruments [36]. The slope/bias correction (SBC) method is a simple and practical method that operates only on the predicted *Y*-values [37]. SBC establishes a univariate linear model between the primary instrument *Y* values and the secondary instrument predicted *Y* values for selected standardization samples. Then the resulting univariate linear model is used to correct the predictions of the multivariate calibration model for secondary instrument data. These methods have proven that they are suitable for the maintenance of the predictive capabilities of multivariate calibration models when there are small changes in instrument or measurement conditions.

The main objective of this research work was to investigate several calibration-transfer strategies, using low-cost handheld NIR transmission spectrophotometers, for the quality control and falsification of medicines marketed in low- and middle-income countries. Firstly, PLS regression models have been developed and validated for the quantitative analysis of metformin drug-based formulations on each device. Secondly, the transferability study of PLS models based on multisource NIR measurements has been assessed using different handheld NIR instruments.

In this study we selected metformin because it is the most common first-line treatment and has become the drug of choice in the treatment of diabetes mellitus 2, despite the arrival of innovative drugs. However, the quality of this drug remains uncertain but potentially of great public health importance in this period of substandard and falsified medicines increase. Several studies on the quality of anti-diabetic drugs have been carried out, among which a few studies have shown an estimated prevalence of 10.8% of fake anti-diabetic drugs in low-income countries [38]. The consequences of fake drugs are harmful to public health causing potentially serious therapeutic ineffectiveness, adverse effects, and drug resistance. Several studies and reports point to the massive presence of poor quality medicines in low income countries due to the presence of inadequate or almost non-existent supply systems and / or quality assurance systems [39].

2. Material and methods

2.1. Chemicals

2.1.1. Pharmaceutical products

The antidiabetic drugs for which the methods were developed included solid dosage forms (tablets) of metformin as described in Table 1. Metformin formulation samples in different tablet dosage forms (500 and 850 mg) were bought in the Belgian pharmaceutical market. These two categories of dosage forms represent most of the metformin formulations produced or marketed globally and most of them are film- coated or coated tablets.

Hypromellose (hydroxypropyl methylcellulose, METHOCEL™ E5 Premium LV) was purchased from Colorcon Ltd (Dartford Kent, England), povidone (Kollidon® 30 LP) was purchased from BASF (Ludwigshafen, Germany), a mixture named “Mix A” containing microcrystalline cellulose (Avicel®) 40%, magnesium stearate 22%, talc 18%, starch 11% and colloidal silicon dioxide (Aerosil®) 9% (w/w)) was given free of charge by Pharmakina Sprl (Bukavu, DRC).

These raw materials represent the most common excipients used in metformin coated tablet formulations. Hypromellose and povidone were added to Mix A to obtain Mix B, in the proportions 1/1/2 respectively. Mix B was then used as a matrix for the standard samples of calibration and validation. The metformin hydrochloride was purchased from Fagron (Nazareth, Belgium).

Table 1 *- Description of analyzed samples.*

Code	Active Pharmaceutical Ingredient	Brand Name	Dosage (mg)	Galenic forms	Batch N°
E1	Metformine HCl	Metformine sandoz	500	Film coated tablets	KX6343
E2	Metformine HCl	Metformine Mylan	500	Film coated tablets	4640240A
E3	Metformine HCl	Glucophage	500	Film coated tablets	E206534
E4	Metformine HCl	Glucophage	850	Film coated tablets	E206167
E5	Metformine HCl	Metformax	850	Coated tablets	8204
E6	Metformine HCl	Metformine sandoz	850	Film coated tablets	KX0454
E7	Metformine HCl	Metformine Mylan	850	Film coated tablets	4650264A

2.1.2. SOLVENTS AND REAGENTS

Analytical grade 37% hydrochloric acid used to prepare the dissolution medium was purchased from VWR (Leuven, Belgium). Demineralized water was obtained with a MilliQ Plus 185 system (Millipore, Molshein, France).

2.2. UV and NIR instrumentations

Ultraviolet absorbance measurements were performed on a UV/VIS spectrophotometer, Perkin Elmer (Norwalk, CT 05859 USA).

NIR measurements were performed in transmission mode. Three low-cost handheld transmission NIR spectrophotometers (NIR-M-T1, Innospectra Corp. coded as NIR-M-T1-A, NIR-M-T1-B and NIR-M-T1-C) were used. Each spectrum corresponds to the average of 32 scans and with a digital resolution of 228, PGA gain of 16 in the range of 900–1700 nm. The lamp was turned ON for 1 h before starting

the analysis to reach a stable detector's temperature and a new background was measured before each sample scan while the lamp remained lit during the whole sample analysis. Standard solutions and test sample solutions were directly scanned in Hellma UV quartz cell, 2 mm pathlength.

The NIR spectrophotometers were verified for wavelength accuracy using the Bruker BRM2065 standard (Bruker Optics, Ettlingen, Germany). The X-axis were recalibrated when necessary to achieve a maximum ± 1 nm wavelength difference.

For reflection analysis of powder raw materials, NIR-S-G1 reflection module (Innospectra Corp.) was used with the same acquisition parameters.

2.3. Preparation of samples

2.3.1. NIR Calibration and validation standards

The calibration and validation standards were reconstituted samples within the excipients matrix containing known concentration of metformin hydrochloride. Calibration and validation standard samples were prepared by dissolving reference metformin hydrochloride in 1.1 M HCl solution. Metformin standard samples were prepared with an adequate amount of matrix (Mix B) to mimic tablet formulations. The acidification of the aqueous solution was used to facilitate the complete dissolution of metformin in the presence of insoluble excipients and gelifying agent such as hypromellose. The regression models were developed using gravimetric data as reference measurements.

Three independent series of samples were realized for both calibration and validation sets with five concentration levels (50, 75, 100, 125 and 150 mg/mL of equivalent metformin hydrochloride) in which the target concentration was 100 mg/mL.

Before analysis, metformin standard solutions were filtered through a 0.45 µm PTFE filter. For each concentration level, three validation standard samples were prepared independently, and three measurements were performed for each sample solution (replicates). The predicted concentrations of the replicates were averaged (n = 3) for validation computations.

2.3.2. Preparation of samples for UV and NIR assays

2.3.2.1. UV standard sample preparation. The sample tablets were analyzed following the ultraviolet spectroscopy method of the "Metformin Hydrochloride Tablets" USP monography [40]. In this case,

standard solution was prepared by dissolving and diluting metformin hydrochloride in water to get a final concentration of 10 µg/mL of metformin hydrochloride.

2.3.2.2. NIR sample assays. An amount of powder equivalent to 200 mg of metformin hydrochloride was diluted in 2.0 mL of 1.1 M HCl solution to get the nominal target concentration of 100 mg/mL. Three measurements were performed for each filtered sample solution and the predicted concentrations were averaged (n = 3).

2.4.Data analysis

MATLAB R2018a (The Mathworks, Inc., Natick, MA, USA) and PLS toolbox Version 8.8.1 (Eigenvector Research, Inc. Wenatchee, WA, USA) were used for chemometric data analysis and computations.

2.4.1. PLS analysis

The PLS model was built for each NIR instrument. Spectral range, preprocessing strategies, and number of latent variables were optimized using the RMSEP as quality criterion.

2.4.2. Validation

The NIR predictive models were validated using the total error approach with the acceptance limits set at ± 5% with a β-risk level of 95%. All validation calculations were carried out with E-noval 4.0b (Pharmalex Belgium, Mont-saint-Guibert, Belgium).

2.4.3. Transferability studies

The deployment of the regression models was determined by global modelling approach and calibration transfer methods as described in Fig. 1. The global modelling approach consisted of pooling the calibration sets of the three devices and then predicting the validation samples in a device-wise fashion. The transformation matrices of different calibration transfer methods (DS and SST) were estimated from the spectra of a primary instrument. SBC performed a univariate linear model between the primary and secondary predicted data; then the linear model (considering the slope and intercept) was used to correct the predicted data from secondary instruments.

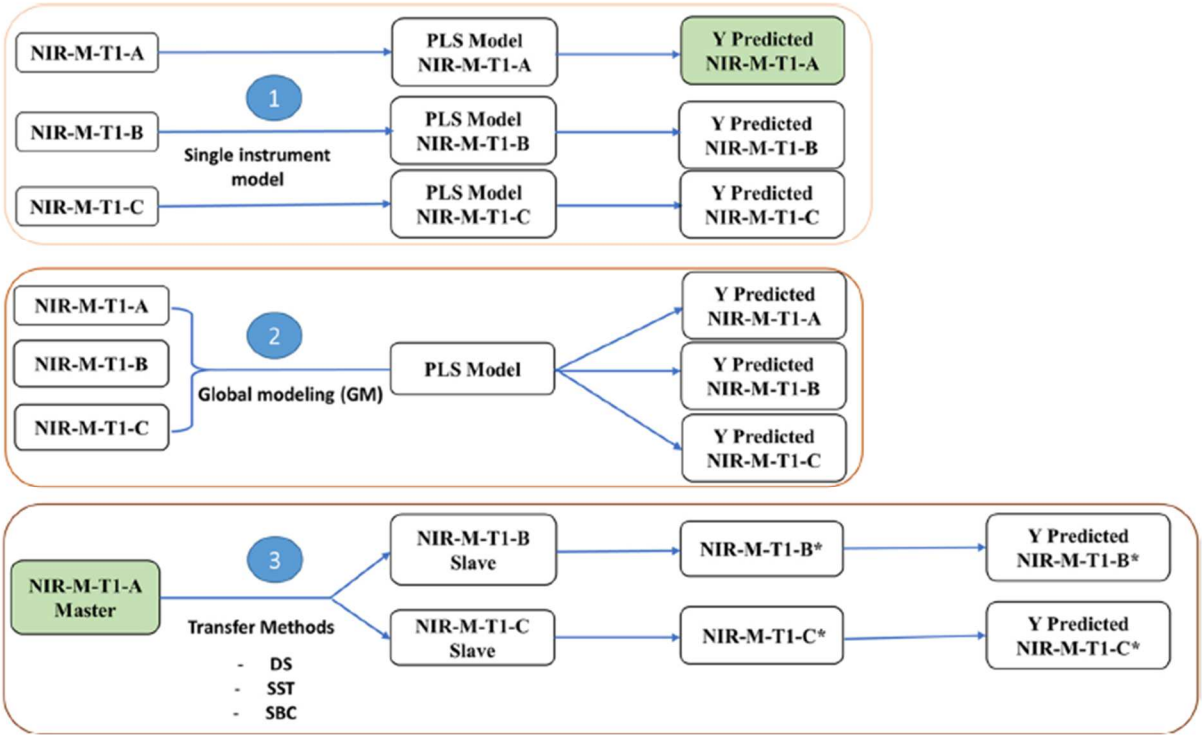
The RMSEP and accuracy profile were used as the performance criteria to assess the transfer capability of calibration model.

3. Results and discussion

3.1. Spectral preprocessing

The metformin spectra recorded with NIR-S-G1 device in reflection (solid state) and with NIR-M-T1-A in transmission (aqueous form) are represented in Fig. 2. Specific spectral features of metformin are present in the spectral range where the absorption of O–H bonds of water is weak (blue shaded area). For the spectrum of metformin in aqueous form no difference is observable with the naked eye.

Figure 1. *(1) The first strategy consisted in developing and validating a PLS regression model on each instrument (NIR-M-T1-A, NIR-M-T1-B and NIR-M-T1-C). (2) The second strategy was to develop a global PLS regression model using data from three instruments and to make data predictions of each instrument on the global model. (3) The third strategy consisted of considering an instrument as primary (NIR-M-T1-A) and performing a transfer to the other two secondary instruments (NIR-M-T1-B and NIR-M-T1-C). The data transferred from each secondary instrument was predicted on the primary instrument model. GM, DS, SST, SBC indicate global modelling, direct standardization, spectral space transformation and slope bias correction; respectively. * indicates "transferred". Diagram representing the different strategies for developing PLS models based on multisource NIR-M-T1 data.*

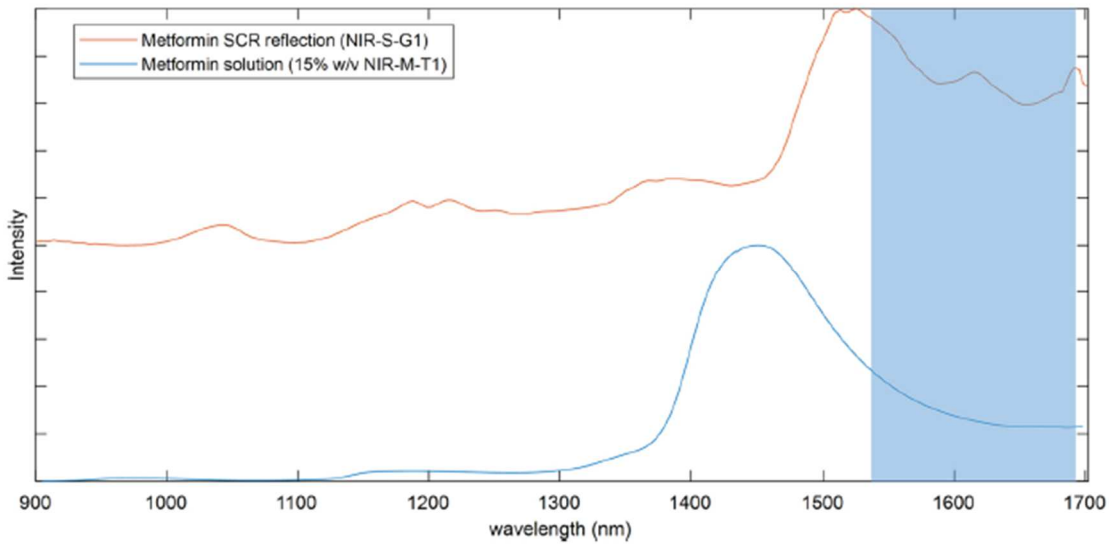


However, chemometric techniques can detect very small differences in absorption. In addition, this spectral range allowed to build PLS models with better prediction performances. In order to reduce variability and enhance chemical spectral features, the raw NIR spectra were preprocessed as described in Table 2. Savitzky-Golay (SG) smoothing and differentiation filter (second-degree polynomial and first derivative) was applied to remove noise and baseline signals. For global modelling, Standard Normal Variate (SNV) were applied to the smoothed and differentiated signals.

3.2.PLS calibration model and validation

The PLS regression models were built based on the spectral data of the standard samples of metformin using the gravimetric data as a reference. Spectral data were acquired from each NIR-M-T1 spectrophotometer. Table 2 shows the applied preprocessing, selected spectral ranges and PLS regression selected parameters.

Figure 2. *NIR reflection spectra of pure raw materials measured with the NIR-S-G1 spectrophotometer (upper spectrum in red) and NIR transmission spectra of metformin solution (lower spectrum in blue) measured with NIR-M-T1 spectrophotometer. The spectra were offset for better visualization. The accessible spectral range of sample solutions is highlighted in the blue shaded area.*



The regression vector of the PLS was compared to the preprocessed transmission spectrum of metformin HCl (after subtraction of the HCl 1 M spectrum) for the NIR-M-T1-A device (**See** Fig. 3). As

one can see, the main features of metformin are in accordance with the regression vector. This comparison can be considered as a confirmation of the specificity of the regression model.

The optimal models were successfully validated for each NIR-M-T1 instrument, using the total error approach. The accuracy profile plots are shown in Fig. 4. The NIR methods also satisfied to the ICH Q2(R1) validation criteria in term or linearity, trueness, accuracy, and precision as shown in Table 3. The limits of quantification, considering the target concentration value at 100 mg/mL, are suitable for the quality control of metformin as well in case of substandard and falsified medicines.

Table 2 *parameters of the PLS regression models.*

Metrics	PLS Models			
	NIR-M-T1-A	NIR-M-T1-B	NIR-M-T1-C	Global Modeling (GM)
Spectral range	1547.91–1691.96 nm	1548.34–1692.09 nm	1549.16–1692.35 nm	1513.87–1689.48 nm
Preprocessing	SG(1,2,7) + MC	SG(1,2,5) + MC	SG(1,2,5) + MC	SG(1,2,7) + SNV + MC
# LVs	5	4	4	7
SG: Savitzky-Golay (derivative, polynomial order, window size)				
SNV: Standard normal variate				
MC: Mean center				

The large dispersion of the relative error of the lowest concentration level can be explained by the fact that, in addition to being close to the lower limit of quantification considering the acceptance limits set according to the US Pharmacopoeia, the matrix used contained hypromellose (HPMC). This excipient has gelling properties increasing the viscosity of the solution, especially for the lowest concentration of metformin (because of the higher excipient/API ratio). This impacts randomly the amount of metformin present in the solution after the filtration step.

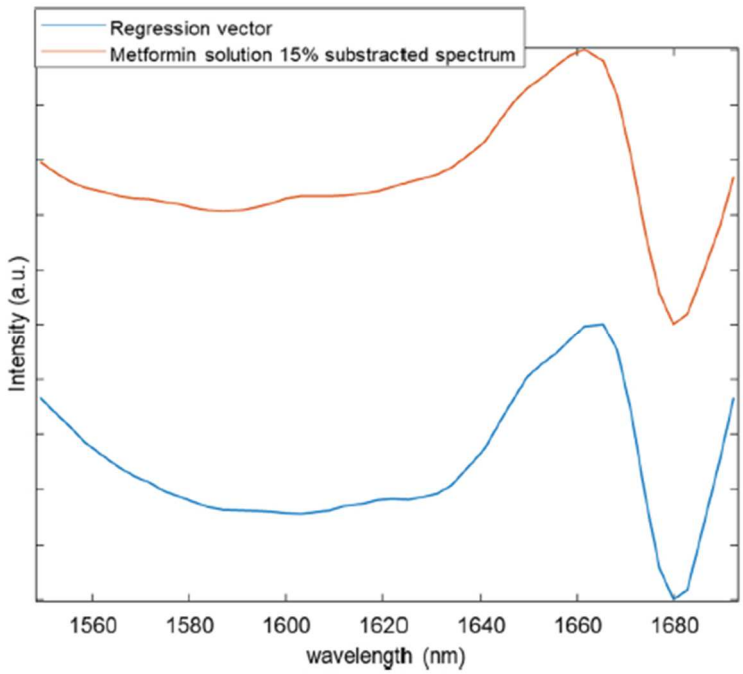
3.3.PLS transfer model

After being developed, the PLS models were tested for their predictive ability on multisource spectral data from secondary instruments. Unfortunately, a bias was observed when the model was developed with NIR-M-T1-A data and used to predict validation samples measured on NIR-M-T1-B and NIR-M-T1-C instruments. Additionally, observing the score plots, the data from secondary instruments are considered as outliers to the model and unfortunately cannot be analyzed directly without correction.

To assess the model transfer capability of handheld NIR instruments, the NIR-M-T1-A instrument was selected as the primary instrument because it gave the best predictive performances and the

best accuracy profile compared to the others. The other two instruments (NIR-M-T1-B and NIR-M-T1-C) were used as secondary instruments. The observed differences in spectral intensity might cause systematic prediction errors when the calibration model built from the primary instrument is used to predict spectra measured with the secondary instruments. To maintain the predictive capacities of the calibration model, global modelling and transfer methods have been applied to mitigate the effects of spectral differences. The transfer capabilities of both two strategies were evaluated and compared by considering some transfer parameters such as spectral interval, number of transfer samples and spectral resolution.

Figure 3. *Regression vector (lower trace) presented with the solution spectrum of Metformin HCl (150 mg/mL) measured with the NIR-M-T1-A spectrophotometer (upper trace). The spectra are offset for better visualization. The spectral range and the preprocessing are the ones of the PLS regression model (NIR-M- T1-A model). The preprocessed spectrum of HCl 1 M was subtracted from the spectrum of the metformin solution to enable the visualization of the metformin bands. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)*



The first strategy applied a global modelling approach which consisted of adding the new variability arising from the supplementary instrumental data in the calibration set by pooling the

calibration datasets of the three instruments. The global model was tested for its predictive performances using validation data sets from all three instruments individually. The results showed that, using the global modelling approach, the PLS model itself can compensate the instrumental variations by adding two more latent variables. The optimal model was tested to predict validation data from three instruments taken individually. The RMSEP value was found to be 1.21, 1.25 and 1.32 mg/ mL for NIR-M-T1-A, NIR-M-T1-B and NIR-M-T1-C respectively; the corresponding accuracy profiles indicated an improvement in predictive performances for multisource spectral data. In some cases, this may be one of the alternative solutions to compensate the NIR spectral variations that may either arise 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. In addition, the handheld NIR-M-T1 instruments are still in the development stage and do not have sealed shells that can protect the system from any variation in temperature and / or humidity. Thus, special attention must be paid for their use in tropical areas where there are strong variations in temperature and relative humidity, depending on the seasons. However, this approach implies the development of a complete calibration set on each new instrument to be used until enough equipment are added in the calibration set to be robust to new equipment variability.

The second strategy was to apply transfer methods to match the signals from the secondary instruments to the primary instrument. Fig. 5 shows the accuracy profiles of the prediction of transferred data on the primary-based PLS model (NIR-M-T1-A); using DS, SST and SBC transferred methods of the data measured on the secondary instruments (NIR- M-T1-B and NIR-M-T1-C). Compared to each other, SST allowed good predictions with only 3 standardization spectra required while DS needed all calibration set (45 samples) to achieve comparable standardization performances.

Figure 4. *A, B and C represent the accuracy profiles of the models obtained for the three validation series with the NIR-M-T1-A, NIR-M-T1-B and NIR-M-T1-C spectrophotometer, respectively; using an acceptance limit of ± 5% and a β-risk level set at 95%. The plain red line is the relative bias, the dashed blue lines are the 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 color in this figure legend, the reader is referred to the web version of this article.)*

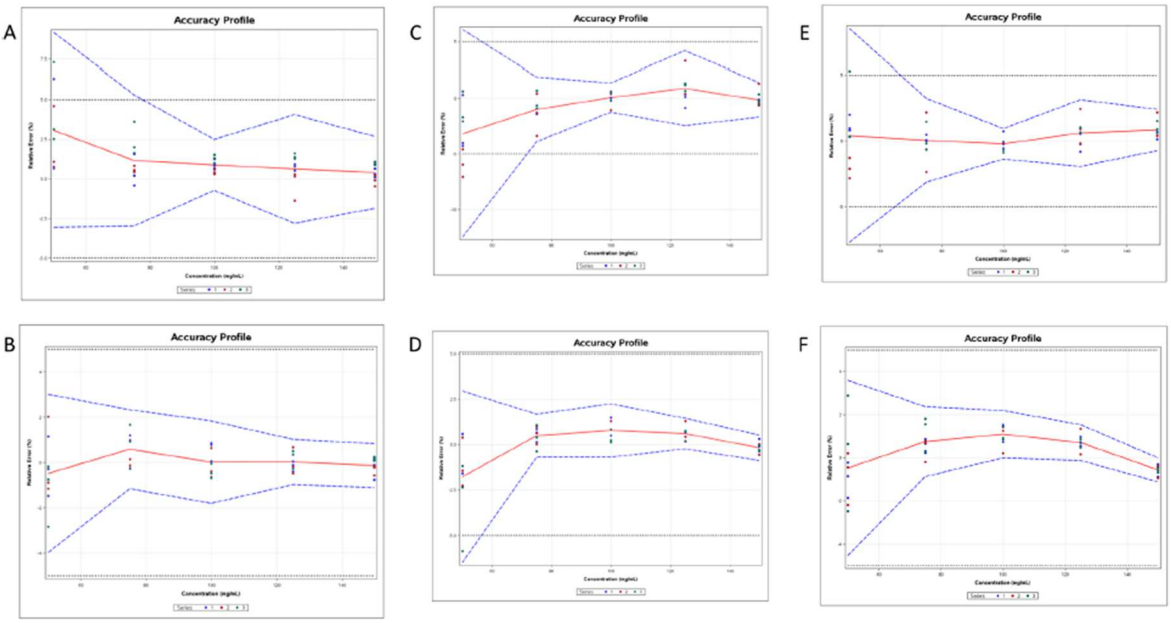


Table 3 - *ICH Q2 (R1) validation criteria values of the PLS regression models.*

	Concentration level (mg/mL of equivalent metformin hydrochloride)	NIR-M-T1-A	NIR-M-T1-B	NIR-M-T1-C	GM
Trueness Relative bias (%)	49.98	-0.07499	1825	-0.5061	1836
	74.93	0.1040	0.5373	0.4333	0.5028
	99.86	0.1600	0.5532	-0.04003	0.2165
	124.8	0.1691	0.8060	-0.08075	0.4533
	149.7	-0.07609	0.2169	-0.3226	0.04383
Intra-assay precision Repeatability (RSD%)	49.98	1004	2551	1903	3103
	74.93	0.4094	0.7589	0.8589	0.8661
	99.86	0.3613	0.2975	0.4602	0.7845
	124.8	0.1329	0.2459	0.3777	0.7224
	149.7	0.2768	0.2472	0.2994	0.9850
Between-assay precision Intermediate precision (RSD%)	49.98	0.5017	1275	0.9510	1551
	74.93	0.4502	0.7568	0.6436	0.6490
	99.86	0.6788	0.4877	0.5611	0.8052
	124.8	0.3137	0.8191	0.4715	0.9019
	149.7	0.4145	0.6952	0.4538	1475
Accuracy Relative β -expectation tolerance limits (%)	49.98	[− 2.531, 2.381]	[− 4.415, 8.066]	[− 5.161, 4.149]	[− 4.664, 8.336]
	74.93	[− 1.759, 1.967]	[− 2.395, 3.469]	[− 1.668, 2.535]	[− 1.312, 2.317]
	99.86	[− 2.286, 2.606]	[− 1.073, 2.179]	[− 1.580, 1.500]	[− 1.492, 1.925]
	124.8	[− 0.7378, 1.076]	[− 1.930, 3.542]	[− 1.005, 0.8433]	[− 1.060, 1.967]
	149.7	[− 0.7533, 0.6011]	[− 1.453, 1.886]	[− 1.068, 0.4226]	[− 2.020, 2.107]

Principal component analysis (PCA) was performed on the spectral data to choose the standardization samples and to verify if they fit into the calibration data set and are not recognized as outliers. This was done by evaluating the Hotelling T^2 and visualizing the score plots. Two extreme points (low and high concentrations) and one central point (target concentration) sample spectra were chosen as standard spectra to build the SST transfer model to ensure a good linear fit. Statistics indicate better accuracy in predicting concentrations of standard samples of metformin HCl in the concentration range from 71.67 to 149.7 mg/mL for NIR-M-T1-B data and 56.37–149.7 mg/mL for NIR-M-T1-C data. Moreover, similar results are observed when NIR-M-T1-C was considered as primary instrument while a strong positive bias was observed when NIR-M-T1-B was considered as primary instrument. There was an important bias when the standardized NIR-B validation data were predicted on NIR-M-T1-A PLS model and NIR-M-T1-C PLS model, respectively.

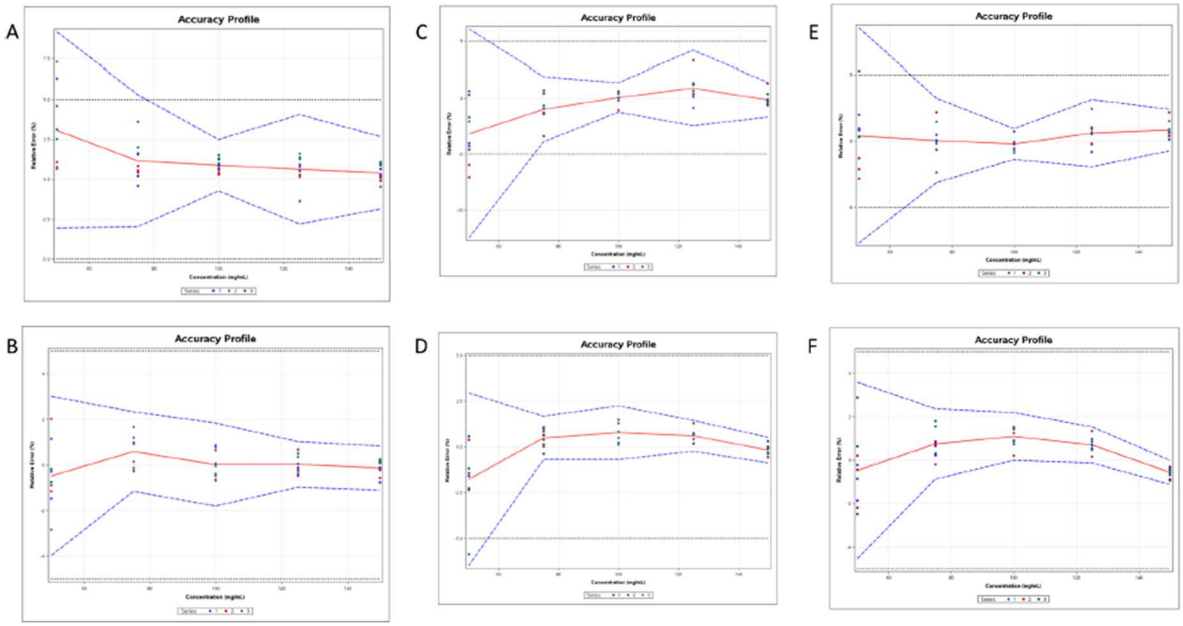
Likewise, the results were not satisfactory enough using NIR-M-T1-B as a primary; especially for the SST algorithm. This can be explained by the fact that there are significant spectral differences between the NIR- M-T1-B instrument and the two other instruments; in terms of which could have a negative effect on the quantitative model. This was confirmed by the manufacturer that indicated that NIR-M-T1-B was slightly less resolved than the other two devices and may need a slit re-alignment.

For the SBC method, the values of R^2 (determination coefficient), slope (the slope for the linear fit) and the intercept, using the predicted data from secondary instruments (NIR-M-T1-B and NIR-M-T1-C) versus the predicted data from primary instrument (NIR-M-T1-A). The SBC allowed to correct the bias observed during the prediction of the standard samples of metformin measured with the secondary instruments on the primary based PLS model. the predicted data thus corrected allowed to successfully validate the primary model in the concentration range of 66.34–149.7 mg/mL with the NIR-M-T1-B data and from 49.98 to 149.7 mg/mL with NIR-M-T1-C data.

In this study, taking into account the results obtained with GM, DS, SST and SBC, it may appear that the SST and SBC remain the most interesting transfer methods because they allow to have a better transfer model with less standardization samples (already with 3 samples with SST and 5 samples with SBC) which is much more practical in various situations where it is important to develop PLS models for several molecules and to deploy them on a large number of handheld instruments. The global modelling strategy is also interesting, but it is limited by the number of instruments available to achieve a robust method. In the frame of the present study, only three

instruments were used. However, it would be interesting to test a higher number of instruments and check when a convergence is achieved indicating the optimal number of devices to include in the global modelling. According to the manufacturer, at least three to five other instruments should be used for the calibration set to test the multisource variabilities, but this number of devices should be confirmed through testing studies. Another approach could be the fusion of calibration models as described by Halberg et al. [41]. This approach considers the progressive clustering of calibration datasets to obtain the minimal number of calibration models to maintain while keeping the best predictive performances.

Figure 5. *Accuracy profiles of NIR PLS method based on the transmission mode, using NIR-M-T1-A as primary instrument. A represents the accuracy profile obtained with NIR-M-T1-B validation data standardized by DS; B represents the accuracy profile obtained with NIR-M-T1-C validation data standardized by DS; C represents the accuracy profile obtained with NIR-M-T1-B validation data standardized by SST; D represents the accuracy profile obtained with NIR-M-T1-C validation data standardized by SST; E represents the accuracy profile obtained with NIR-M-T1-B validation data corrected by SBC; F represents the accuracy profile obtained with NIR-M-T1-C validation data corrected by SBC. The acceptance limit set at ± 5% with a β-risk level set at 95%. The plain red line is the relative bias, the dashed blue lines are the 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 color in this figure legend, the reader is referred to the web version of this article.)*



SBC was applicable since it applies on threes instruments of the same brand and model but, due to its univariate nature, it could not effectively model spectral differences resulting from instruments with different configuration. This is one limitation of the SBC method.

The transfer strategy with the SST method seems promising for the case of different types of spectrophotometers. SST provides satisfactory prediction characteristics and better validation criteria values comparable to GM from spectroscopic measurements subjected to instrumental change, using only 3 representative standardization samples that were run on both primary and secondary instruments and were sufficient to support the model transfer between the two instruments. It would then be sufficient to assess the degree of variation between these portable instruments and determine their performance for transmission mode analysis.

3.4.Assay

The validated PLS models were then used to analyze seven formulations of metformin tablet dosage forms from different manufacturers. The samples were of different brands with two main dosage tablet forms (500 mg and 850 mg metformin hydrochloride film-coated tablets). In addition, all samples were also analyzed using the USP UV method used as quantitative reference method. The average results per sample are shown in Table 4. All samples were found to be within the acceptance limits of the reference method, i.e. ± 5.0% of the declared content value even after calibration transfer. Compared to the UV spectrophotometric method, the results obtained were similar and showed that the models developed could be used to screen most dosage forms of metformin tablets for substandard medicines.

4. Conclusion

Considering the aim of this study, we successfully developed and validated the PLS models based on handheld NIR transmission measurement for the analysis of metformin-based tablet formulations. These models were used to quantify metformin in different tablet formulations which were found to be compliant to the USP reference method. The transferability study of developed PLS regression models was assessed using multisource spectral data to ensure that the PLS model generated on one instrument will work on another one. Global modelling approach (GM), standardization algorithms (DS and SST) and slope/bias correction method were applied and

compared to each other. GM, SST and SBC were found to be effective in managing the spectral variations by correcting or removing the spectral drifts. The GM strategy balances the different sources of variability, which allows for a robust model.

Table 4 *Assay results for metformin samples with UV/VIS and NIRS Methods. The drug content results are presented in percentage (%) of the stated amount of active ingredient (acceptance limits +/- 5.0%).*

Samples	UV	NIR-MT1-A	NIR-MT1-B	NIR-MT1-C	SST (NIR-MT1-B)	SST (NIR-MT1-C)	SBC (NIR-MT1-B)	SBC (NIR-MT1-C)
	% of claimed value (95,00%–105,00%)							
E1	99,68	98,31	105,25	96,67	103,69	97,41	101,24	101,41
E2	97,10	97,48	102,60	99,14	102,25	99,23	98,57	100,45
E3	101,01	98,00	101,22	98,54	101,49	98,42	98,62	99,27
E4	101,16	97,50	102,06	98,10	101,49	97,75	98,07	98,84
E5	99,94	95,76	100,62	98,01	99,04	98,15	96,11	99,93
E6	102,46	96,63	100,59	97,00	100,54	97,55	97,74	99,04
E7	100,25	97,88	101,90	97,97	102,38	98,08	98,83	99,31

Nevertheless, some calibration transfer difficulties were encountered due to lack of information on the variations in spectral responses between the different NIR-M-T1 handheld spectrophotometers available on the market. However, it would be interesting to extend this study to a higher number of NIR-M-T1 instruments to correct the measurement uncertainties that may arise from the instrument variability and verify their robustness under different environmental conditions.

This constitutes the limitations of our research, but nevertheless the results are very encouraging in the context of a qualitative and quantitative screening of metformin-based pharmaceutical products and allow us to consider their deployment in the field after a robustness study.

CRediT authorship contribution statement

P.H. Ciza: Conceptualization, Methodology, Investigation, Formal analysis, Writing – review & editing. **P.-Y. Sacre:** Conceptualization, Methodology, Writing – review & editing. **C. Waffo:** Investigation. **T.M. Kimbeni:** Writing – review & editing, Funding Acquisition. **B. Masereel:** Writing – review & editing, Funding acquisition. **Ph Hubert:** Writing – review & editing, Funding acquisition. **E. Ziemons:** Conceptualization, Methodology. **R.D. Marini:** Conceptualization, Methodology, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ciza Patient reports financial support was provided by

Academy of Research and Higher Education. Sacre Pierre-Yves reports financial support was provided by Fund for Scientific Research.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jpba.2022.114755.

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