**Benchtop NIR data standardization on handheld spectrometers to identify paracetamol tablets on the Belgian market with SIMCA.**

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Near infrared spectroscopy (NIRS) allows innovative applications in terms of quality control, e.g. in raw materials verification, in process analytical technology (PAT) and in discrimination between genuine and falsified medicines. The development of small and cheap handheld devices is expanding in the field, while trying to keep similar performances as benchtop instruments have. Considering traceability and quality control of drug compounds, this work is intended to identify 13 different paracetamol tablets on the Belgian market by using NIRS. The performances of a FT-NIR benchtop and two handheld NIR spectrometers were investigated comparatively. All spectra were collected through the blister in the reflectance diffuse mode. NIR spectral fingerprints were pretreated and analyzed using Principal Component Analysis (PCA) and classified with Soft Independent Modeling of Class Analogy (SIMCA). The performances of the spectrometers were evaluated after standardization of the reference benchtop database to the handheld spectrometers. The instrumental response of the benchtop spectrometer was standardized towards the handheld device using the Piecewise Direct Standardization (PDS) algorithm. These investigations permitted the advantages and limitations of NIR data standardization on predictive models to be pointed out. This strategy purposes to highlight potential of using a large reference database collected by NIRS for pharmaceutical analyses by handheld NIR spectrometers in terms of traceability but also as quality control during the production of medicines or detection of illegal medicines.

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Benchtop NIR data standardization on handheld spectrometers to identify paracetamol tablets on the Belgian market with SIMCA.

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Abstract

Near infrared spectroscopy (NIRS) allows innovative applications in terms of quality control, e.g. in raw materials verification, in process analytical technology (PAT) and in discrimination between genuine and falsified medicines. The development of small and cheap handheld devices is expanding in the field, while trying to keep similar performances as benchtop instruments have. Considering traceability and quality control of drug compounds, this work is intended to identify 13 different paracetamol tablets on the Belgian market by using NIRS. The performances of a FT-NIR benchtop and two handheld NIR spectrometers were investigated comparatively. All spectra were collected through the blister in the reflectance diffuse mode. NIR spectral fingerprints were pretreated and analyzed using Principal Component Analysis (PCA) and classified with Soft Independent Modeling of Class Analogy (SIMCA). The performances of the spectrometers were evaluated after standardization of the reference benchtop database to the handheld spectrometers. The instrumental response of the benchtop spectrometer was standardized towards the handheld device using the Piecewise Direct Standardization (PDS) algorithm. These investigations permitted the advantages and limitations of NIR data standardization on predictive models to be pointed out. This strategy purposes to highlight potential of using a large reference database collected by NIRS for pharmaceutical analyses by handheld NIR spectrometers in terms of traceability but also as quality control during the production of medicines or detection of illegal medicines.

Keywords : Quality control, SIMCA, Standardization, Near infrared spectroscopy, Handheld devices.

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1. Introduction

Near infrared spectroscopy (NIRS) is commonly applied for the quality control of pharmaceuticals, cosmetics and food supplements, among other [1–3]. Tablets are the most commercialized health products in the world and are strongly subject to illegal manufacturing or fraud [4,5]. Quality controls are performed to guarantee the conformity of the product during production and distribution and to ensure quality control in terms of traceability [6–12]. Near infrared devices are also well studied to evaluate the conformity of raw materials and pharmaceuticals [13–15]. Several publications and special issues have pointed out the interest in using NIRS to detect sub-standard and falsified medicines [16–21]. There is a broad interest for NIRS because it is a non-invasive and non-destructive method allowing rapid analyses without the use of solvents. It also permits avoiding direct contact with the material to guarantee safety for the analyzer. Recording a NIR spectrum provides a fingerprint which, when combined with appropriate chemometric tools, allows numerous applications in pharmaceutical analyses [22,23].

Over the last years, NIR handheld devices have become increasingly popular. In fact, these miniaturized instruments are portable and are commercially available at lower cost compared to benchtop instruments [24–27]. In the industry, NIRS is commonly used to generate classification models to ensure quality control during the production stage while creating a reference database with the accumulated NIR spectra. The development of a classification model needs many samples and requires the use of only one device for referencing. In case of collecting data in pharmacies or in distribution networks or to ensure traceability of pharmaceuticals, the use of a handheld equipment is best suited. Therefore, it appears to be of interest to have access to a large set of data obtained on an instrument considered as reference data (such as a benchtop device) allowing subsequent transfer of the information to other instruments as standardization. Once the reference database has been standardized, it could be used by both benchtop and handheld devices for new sample identification.

Bouveresse et al. studied standardization methods for the correction of the differences between the instrumental responses of NIR spectrometers, when using calibration models for prediction [28,29]. Direct standardization and Piecewise Direct Standardization (PDS) are commonly applied [30–33]. The critical steps are highlighted in several references [34–37]. Standardization of NIR data for classification purpose is less described in the literature. Nevertheless, the use of handheld devices and benchtop databases requires standardization before spectra can be compared. The potential of NIRS and Raman spectroscopy is thoroughly described in the literature to study the qualitative and quantitative composition of tablets, even when measurements are performed through the blisters [8,14,38–44].
In this paper, Soft Independent Modelling of Class Analogy (SIMCA) was applied to identify paracetamol tablets on the Belgian market by NIRS with handheld spectrometers using benchtop data standardization. Two handheld devices were studied which differentiates by size, price, and spectral acquisition range. The performance of both instruments to obtain a characteristic fingerprint for the paracetamol tablets in their blister package was evaluated. Thirteen different tablets from ten manufacturers and issued from different batches were analyzed. Differences reside in the paracetamol/tablet weight ratio, in their shape, in the dose of paracetamol and in the blister composition. The results obtained with the handheld devices were compared with those of a benchtop instrument.

The spectral data was transferred from the benchtop instrument to the handheld devices using the Piecewise Direct Standardization (PDS) algorithm. Standardization was performed for each of the thirteen tablets individually. The SIMCA classification model built on the standardized benchtop data was used for classification of NIR spectra recorded with the handheld devices.

Moreover, the performance of the SIMCA model was investigated by adding newly collected data from the handheld devices to the standardized benchtop database. For each handheld instrument, four SIMCA models were created: one using the standardized benchtop spectra and three models using the standardized benchtop spectra and an increasing number of handheld spectra. Statistical parameters used to evaluate the SIMCA models were the sensitivity, the specificity, the error rate and the accuracy.

The study presented in this work aimed to highlight the potential of handheld devices to discriminate pharmaceutical formulations, using a standardized reference database. Indeed, the access to a large reference database created on a benchtop NIRS appears to be a very interesting tool for users of handheld devices. This strategy can be applied during pharmaceutical production processes and in the detection of falsified and illegal medicines.

2. Material and methods

2.1. Drug samples

Formulations of paracetamol were obtained from dispensaries in Belgium. They were collected from different manufacturers. Two different batches were analyzed for each formulation. Paracetamol tablets were selected in this study mainly because of their high amount of active principal ingredient (API) and low amount of excipients resulting in a high ratio of API/tablet weight. This makes the discrimination and identification of these pharmaceuticals by NIR analysis more challenging compared to pharmaceuticals containing a large number or compounds. Furthermore, they are present on the
market in a variety of shapes, doses and blister compositions. Thirteen formulations of paracetamol were selected to obtain a representative set of samples comprising several commercially available formulations (Table 1).

2.2. NIR spectrometers

2.2.1. Frontier FT-IR/NIR

The Perkin Elmer frontier system (UK) can be used as a FT-MIR and FT-NIR benchtop spectrometer. This instrument operates in the range from 15, 800 cm\(^{-1}\) to 4000 cm\(^{-1}\) (632 nm to 2500 nm) in the NIR mode with a fixed data interval of 1 cm\(^{-1}\). The selected spectral range from 7550 cm\(^{-1}\) to 4170 cm\(^{-1}\) contains 1776 data points. An optical fiber probe was used to collect NIR spectra through the blister package. This procedure could be used in routine quality control. The light source is a tungsten-halogen NIR source, and the reflected light is captured by a Deuterated Triglycine Sulfate (DTGS) detector. The optical resolution was fixed to 4 cm\(^{-1}\) (0.4 nm) with an average of 16 scans for each spectrum collected (apparatus dimension: 480 x 570 x 260 mm, 30 kg).

2.2.2. MicroPHAZIR\textsuperscript{TM} RX analyser

The handheld device n°1 (H1) corresponded to the MicroPHAZIR\textsuperscript{TM} RX analyzer (Thermo Scientific\textsuperscript{TM}). It is a ready-to-use apparatus for sample analysis in the field, e.g. for the identification of raw materials in quality control. The parameters for data acquisition were set by default: a spectral range from 6270 cm\(^{-1}\) to 4170 cm\(^{-1}\) (1600 nm to 2400 nm) and 100 data points with a non-fixed data interval. The light source is a tungsten bulb and the diffuse reflected light is captured by a Gallium Indium Arsenide detector (InGaAs detector) with an optical resolution of 12 nm. Each spectrum is an average of 5 scans with a maximum pixel spacing of 8 nm (apparatus dimension: 254 x 292 x 152 mm, 1.8 kg).

2.2.3. NIR-S-G1

The handheld n°2 (H2) corresponded to the handheld devices S-G1 NIR from INNOSPECTRA Corporation. It is also a ready-to-use device. The acquisition parameters were fixed by the manufacturer in the spectral range from 11 111 cm\(^{-1}\) to 5882 cm\(^{-1}\) (900 nm to 1700 nm) with a non-fixed data interval. The spectral range from 7550 cm\(^{-1}\) to 5882 cm\(^{-1}\) was selected for this study, corresponding to 116 data points. It operates in a reflective mode with two integrated tungsten halogen lamps coupled to an InGaAs detector. Only 1 scan is collected for each spectrum with a spectral resolution of 10 nm (apparatus dimensions: 82 x 63 x 40 mm, 0.136 kg).
2.3. Data acquisition

All spectra were recorded in the reflectance diffuse mode through the blister (transparent or opaque). For each device, a data matrix of 520 spectra was measured: 13 formulations, 2 batches for each formulation and 20 tablets for each batch.

2.4. Chemometric methods

2.4.1. Data pretreatment

Variation within individual NIR spectra is the result of the physical characteristics and the chemical composition of the sample. Additive and multiplicative effects are due to scattering or pathlength effects which reflect physical rather than chemical properties of the sample [45]. The NIR spectra were pretreated to eliminate some of the differences between them due to physical characteristics. Several mathematical transformations such as the Standard Normal Variate transformation (SNV), detrending, or derivative methods are often used for this purpose [46]. Derivative methods permit small differences between similar spectra to be enhanced. The first derivative was applied using the Savitzky and Golay algorithm [47] with a second order polynomial and a window size adapted for each spectrometer. For the benchtop spectra, the window was set at 17 data points to provide maximal information in the spectra. Windows of 5 and 7 data points were selected for handheld 1 and 2, respectively. Subsequently, SNV [46,48,49] was applied on the first derivative data to remove the multiplicative effects. The combination of the derivative method and SNV has been described in recent papers [14,50]. Mean centering was applied before Principal Component Analysis (PCA).

2.4.2. Exploratory data analysis by Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is commonly used to perform exploratory data analysis of spectral data [10,38]. PCA reduces the dimensions of the original data space into latent variables called principal components (PCs) used to highlight the variability in the data. In this study, the three first PCs were used to show similarities and differences between spectra. The spectra are represented as points in the space defined by two or three principal components. The PCs keep the spectral information of the original data space and explain the major part of the variability. In conclusion, PCA was applied to investigate the clustering tendency and define classes for supervised classification.

2.4.3. Selection of a test set for external validation of classification models

The data were split into a training and a test set using the Duplex algorithm [36]. The two samples with highest Euclidean distance between them in a PCA space were selected and placed in a
first set. The next two samples with the highest distance were placed in a second set. This procedure
continued until a predefined number of samples was selected in the test set. The first set and the
remaining samples not selected composed the training set. The training set was used to generate the
classification model, the test set was selected to perform an external validation of the classification
model created.

2.4.4. Linear interpolation of the spectral data

The primary instrument (benchtop) used in this study allowed measurements in a larger
wavelength range including a higher number of data points per unit distance compared to the
secondary one (handheld devices). Therefore, a linear interpolation of the data was performed to
adapt the spectral region and the number of data points between the primary and secondary
instrument before Piecewise Direct Standardization (PDS).

2.4.5. Piecewise Direct Standardization (PDS)

The Piecewise Direct Standardization algorithm allows the NIR spectra for samples measured
on the primary instrument (benchtop) to be transferred to the secondary instrument (handheld). The
response measured at a precise wavelength on the handheld instrument is related to the responses
measured in a small window around the same wavelength on the benchtop instrument. Linear
regression models are obtained between the responses of the handheld instrument and the
corresponding windows on the benchtop instrument. In this study, the moving spectral window was
composed of five wavelengths. Linear regression models are used to predict the response of the
handheld instrument based on the responses measured on the benchtop instrument within a defined
spectral window. PDS corrects for the differences between the instrumental responses of both
instruments [51-53]. The standardization algorithm adapts the benchtop spectra as if they were
measured on the handheld device. These adaptations consist in an alignment of the spectral bands and
also impact the intensity and the widening of the spectral bands in the standardized spectra [32, 35,
45, 52, 54, 55].

2.4.6. Soft Independent Modelling of Class Analogy (SIMCA)

SIMCA is a supervised classification technique that uses samples with known origin (training
samples) to derive a classification rule which allows one to classify new samples (test samples) with
unknown origin in one of the classes. SIMCA considers different classes which are modelled individually
by a separate principal component (PC) model. Two critical values were taken into account to
determine boundaries around the samples belonging to one particular class: The Euclidean distances
towards the SIMCA model assessed by the residual Q statistic and the Mahalanobis measured in the space of the scores and assessed by the Hotelling $T^2$ statistic [56–58]. The position of a new sample was calculated using the scores and the loadings of the PCA model created. If the sample was situated within the restricted space of a training class, then the object was assigned to that class. Confidence limits were set at 95%.

The model complexity was determined by venetian blinds cross validation (VBCV). Cross validation is applied for the validation of the model using the training set of samples. The samples selected by venetian blinds are predicted using the remaining samples in the training set (internal validation of the model).

The optimal number of PCs for each class was selected to obtain a SIMCA model with highest sensitivity, specificity, and smallest error rate. The sensitivity of the SIMCA model is the ability to correctly assign a sample into the right class. The specificity corresponds to the capability of the model to reject samples from all the other classes. The non-error rate (NER) is the average of the class sensitivities and the error rate (ER) is given by $1 – \text{NER}$. The accuracy of the model is defined as the ratio of the correctly assigned samples to the total number of samples.

Predictions were performed for samples which were used to build the model (with venetian blinds cross validation, internal validation) and for samples which were not used to create the model (external validation). The performance of the model was evaluated using the following statistical parameters: the sensitivity, the specificity, the error-rate and the accuracy [58–60].

2.5. Software

All data treatments were performed by using Matlab version R2016b (The Mathworks, Natick, USA). The algorithms of PCA, Duplex, SNV, Savitzky and Golay [61] were part of the ChemoAC toolbox (Freeware, ChemoAC Consortium, Brussel, Belgium, version 4.1). The SIMCA toolbox was downloaded from the Freeware Classification toolbox, version 5.3 [58]. The PDS algorithm was applied using the PLS-toolbox software (version 8.6.1, Eigenvector Research) [55].

3. Results & discussion

3.1. Data processing

In this study, each paracetamol tablet was considered as a class in the SIMCA model (class 1 to class 13). Three spectral data sets were recorded (one on the benchtop and one on each of the two handheld devices). The performance of the SIMCA classification was evaluated on each pretreated spectral data set individually. Each model was evaluated using a set of 130 spectra, selected with the
Duplex algorithm from the respective set of 520 spectra. The aim was to compare the quality of the NIR fingerprints to identify the 13 paracetamol tablets.

In a second step, the benchtop spectral database was standardized to the handheld instruments using the PDS algorithm. For this second step, spectra were pretreated (first derivative followed by SNV) after the linear interpolation step and before the standardization. For each of the thirteen products under study (classes) a transfer model was created using 10 spectra measured on the primary (benchtop) and secondary (handheld) instrument. In total thirteen independent transfer models were obtained. The 10 spectra from the benchtop and handheld device were selected with the Duplex algorithm. Ten spectra correspond to a quarter of the total amount of spectra for each paracetamol formulation. PDS was applied with a window size of five wavelengths. Each of the thirteen transfer models was applied on the corresponding class of benchtop spectra.

The SIMCA classification model was built using this standardized reference database. All the SIMCA models were evaluated using the same set of 130 handheld spectra. The remaining set of 390 handheld spectra was used to increase the number of samples in the SIMCA model. Each time a set of 130 spectra was selected into the set of handheld data with the Duplex algorithm and added to the SIMCA model. Four SIMCA models were built: model 1 based on the 520 standardized benchtop spectra. The models 2 – 3 and 4 were based on the 520 standardized benchtop spectra with 130, 260 and 390 handheld spectra added, respectively. This strategy is summarized in Fig 1.

3.2. Blister package interference and characteristic spectral regions

Preliminary studies were performed with the benchtop to investigate the influence of ambient light, sample orientation and positioning of the probe on the blister of the sample. The influence of the sample orientation on the spectral reading was not neglectable for portable instruments as was the ambient light influence. The data sets collected by both portable instruments contain high variabilities compared to the benchtop one. The PCA plots show more dispersion of the samples in the clusters of the portable instruments compared to the benchtop instrument. This higher variability is attributed to the large optical diameter of the handheld instruments compared to the optical diameter of the benchtop fiber probe.

Spectral bands due to the blister were identified in Figure 2. These spectral bands were present for each of the studied tablets and were more pronounced for the opaque blister than for the transparent one. Between 4500 and 5700 cm⁻¹ and above 6100 cm⁻¹, the spectral bands due to the physical and chemical characteristics of the tablets were observed. The spectral information for tablet and blister were used for identification of the formulation. The mean spectrum for each class (n=40) measured on the three devices were plotted for comparison in Figure 2.
3.3. Individual data investigations

For each device, PCA was applied on the pretreated spectra: 1st derivative followed by SNV and mean-centering. Figure 3 shows the PCA score plots in the space spanned by the principal components PC2 and PC3. The PC1-PC2 and PC1-PC3 plots are shown in the supplementary data.

The first three principal components explain more than 97% of the total variance in the spectra recorded for each device. A cluster of spectra was observed for each paracetamol product. The classes containing the opaque blister information (C9, C11, C12 and C13) were clearly separated from those with a transparent blister. The formulations C3 and C4 had similar excipients and paracetamol/tablet weight ratio. Moreover, these formulations came from the same producer. Consequently, one can assume that these products have the same spectral fingerprint. Nevertheless, a slight separation between the clusters of these two formulations was observed in the score plots. Probably this discrimination was due to differences in the batches of these tablets, small differences in blister composition or differences in physical aspects such as the tablet size, shape, or compaction. Therefore, these formulations were considered as two separated classes in the SIMCA model. In terms of clustering, the three devices seem to provide enough information to highlight differences and similarities between the paracetamol formulations. Based on the clustering tendency, a SIMCA classification was applied to each spectral data set.

The test set for the SIMCA model was selected using the Duplex algorithm: 130 samples were selected for external validation. The remaining 390 samples formed the training set used to construct the model and to perform internal validation. For each class, the optimal number of PCs was selected using venetian blind cross validation.

The SIMCA classification is summarized in Table 2. Confusion matrix is shown in the supplementary data for each of the three models (external validation only).

The SIMCA models for the benchtop and handheld devices H1 and H2 gave a good accuracy for the internal and external validation. The results for the benchtop were slightly better than those obtained with the handheld instruments. For the benchtop, the accuracy was 100.0% for the training set and 99.2% for the test set (129/130). Only one sample from C4 is misclassified as a C3 sample.

Between the handheld devices, the results were slightly better for H1 than for H2. For H1, the accuracy was 99.7% (389/390) for the training set and 97.7% (127/130) for the test set. For the training set a sample from C3 was classified as C4 and for the test set one sample of C2 was classified as C10, one sample of C4 as C2 and one sample of C4 as C7. The minimum accuracy was observed for handheld...
H2: 99.7% (389/390) for the training set and 96.2% (125/130) for the test set. The misclassifications were for the training set: 1 sample of C12 as C9 and for the test set: 1 sample of C2 as C1, 2 samples of C4 as C3, 1 sample of C8 as C4, 1 sample of C12 as C9.

The miniaturization of NIR devices is often linked to the loss of spectral quality giving a poorer discrimination and consequently leading to a slightly lower accuracy. The studied classification parameters such as the error rate, class specificity and sensitivity showed a satisfactory performance (Table 2).

3.4. Standardized data investigations

Two standardized reference data sets were obtained (one for each handheld device). Linear interpolation and standardization were applied on each class and for both handheld devices. The spectral data transferred from the benchtop instrument towards handheld device 1 (BH1) shows similar spectral features as the spectra measured on handheld device 1 (H1). The same observation was made for the transfer of the benchtop spectra to handheld device 2. Figure 4 shows the benchtop spectra, handheld spectra and transferred spectra.

Figure 5 shows the result of the interpolation and PDS transformation on the spectral data for class C1. The black spectrum is the spectrum measured on the benchtop instrument. After interpolation, the red spectrum is obtained, which shows the same major spectral band, however the small spectral band around 5400 cm\(^{-1}\) disappears. The intensity and position of the spectral bands in the red spectrum are adapted by the PDS algorithm which results in the blue spectrum. The blue spectrum coincides with the spectrum measured directly on the handheld device H1 (green spectrum).

PCA was applied to the transferred data and the score plots were compared with those obtained for the handheld devices. The PCA plots obtained for the transferred reference data gave a similar clustering tendency as those obtained for the spectral data measured on the handheld instruments. Figure 6 shows the PC2-PC3 lots for the transferred data. The plots show less variability in the clusters and a better discrimination between classes compared to the plots obtained for the handheld devices. The PC1-PC2 and PC1-PC3 plots are shown in the supplementary data.

To simulate a reliable quality control strategy, we considered that new samples measured by a handheld instrument could be identified by the classification model based on the transferred reference data set. The new spectra collected can be predicted based on the information of the transferred reference database and thereafter they can be added to the SIMCA model. The models were built on an increasing set of spectral data collected with the handheld instrument to confirm the possibility of generating a model with higher accuracy, sensitivity and specificity. The Duplex algorithm was used to
select 130 samples as test set. The same test set was used for the external validation of the four SIMCA models. The remaining 390 samples were used to increase the number of samples in the SIMCA model gradually by 130 samples. The 130 samples were each time selected with the duplex algorithm from the remaining samples. SIMCA model 1 (M1) is based on the 520 standardized benchtop spectra, model M2 – M3 and M4 are based on the 520 standardized benchtop spectra with 130, 260 and 390 handheld spectra added. Confusion matrices are shown in the supplementary data for each of the four models (external validation only).

The SIMCA classification models for handheld device H1 are summarized in Table 3. As shown in Table 3, the accuracy of the SIMCA model 1 (M1) built on the transferred reference benchtop spectra was 99.6% (518/520) for the training set and 97.7% (127/130) for the test set. The misclassifications for the training set are: 2 samples from C3 as C4 and for the test set two samples from C4 as C2 and one sample from C6 as C2. These results were comparable with those obtained initially with the H1 model (see Table 2). The accuracy of the models M2, M3 were comparable to the first model (M1). However, after addition of 390 spectra from H1 (M4), the accuracy increased slightly.

The sensitivity and specificity of the SIMCA models M1-M4 show a maximum of 1 for most classes. For the classes of paracetamol tablets which come from the same manufacturer: C1-C2, C3-C4 and C5-C6, the sensitivity and specificity values were slightly lower than 1. The smallest values were obtained for the C3 and C4 classes. These two formulations were similar in excipients and paracetamol/tablet weight ratio. Nevertheless, it was still possible to discriminate these products by the NIR fingerprint approach combined with SIMCA classification. Probably the discrimination was due to differences between the batches. Confusion matrices for the four models are shown in the supplementary data (external validation only).

The SIMCA model M1 for handheld H2 device (Table 4) shows an accuracy of 100.0% for the training set and 96.2% (125/130) the test set. The misclassified test samples are: two samples from C4 as C3, one sample from C3 as C4, one sample from C7 as C5 and one sample from C8 as C4. The addition of handheld H2 spectra into the model (M2, M3 and M4) improved the accuracy slightly to 97.7% (127/130) for the test set. The sensitivity and the specificity values show a maximum value of 1 for most of the classes. Only classes C3 and C4 show slightly lower values.

The SIMCA models based on the transferred reference data performed well for the classification of the spectra recorded on both handheld instruments H1 and H2. The use of NIRS and SIMCA models created on the transferred reference database proved to be suitable to identify pharmaceutical formulations with handheld instruments.
4. Conclusion

The potential of NIRS using a benchtop or a handheld instrument to identify paracetamol tablets on the Belgian market in their blister package for traceability and quality control purposes was pointed out. Discrimination between samples was achieved based on primary packages, doses, manufacturers, batches and excipients. The SIMCA models built on the spectral data obtained with each of the three NIR devices studied showed the potential of NIRS and chemometrics for the classification of a large representative test set of paracetamol samples. For the SIMCA models based on the individual spectral data of the benchtop (B), handheld 1 (H1) and handheld 2 (H2) instruments, an accuracy of 99.2%, 97.7% and 96.2% was obtained for the test set, respectively. This means that for the test set of 130 spectra only 1 spectrum for the B model, 3 spectra for the H1 model and 5 spectra for H2 model were misclassified. Due to the miniaturization of the instruments, the spectral fingerprints contain less information and reduces the accuracy of the created SIMCA models.

The three NIR devices studied allowed discrimination between products C3 and C4, which have the same excipients, the same paracetamol/tablet weight ratio and which originate from the same producer. The discrimination between these products was probably due to differences in batch, physical aspects such as tablet size, shape or compaction.

The access to a large reference database created on a benchtop NIRS appears to be very interesting for users of handheld devices. The development of a reference database which can be transferred to users of NIR handheld instruments is a powerful and interesting tool for the quality control of pharmaceuticals. The classification model built on the standardized reference spectra can be used to classify new samples measured in the field with the handheld instruments. The importance for the user of the database is that the time-consuming process of collecting spectra to create a new model for identification can be omitted. The prediction of spectra measured on the handheld device H1, using the SIMCA model of the handheld device, resulted in an accuracy for the test set of 97.7 % (3 out of 130 spectra were misclassified). The same accuracy was obtained for the predictions of the test set with the model of the transferred benchtop spectra. However, adding the handheld spectra in the model of the benchtop spectra, improved the accuracy from 97.7% to 98.5%. These results confirm that applying a handheld device can benefit from the information provided by a large reference data base to perform predictions of new samples.

For the H2 instrument, the accuracy of the model obtained with handheld H2 spectra and transferred benchtop spectra was the same: 96.2 % (5 out of 130 spectra were misclassified). An improvement of the accuracy from 96.2 % to 97.7 % (3 out of 130 samples) was observed for the test set samples after adding 390 handheld spectra.
For both handheld devices newly measured spectra were successfully predicted with the SIMCA model based on a large reference data base. The reference data is measured on a centrally located benchtop instrument in the lab. The users of handheld devices can share the same reference data base using each their “personal key” or SIMCA model.

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References


Figure 1: Summary of the analytical strategy.
Figure 1: NIR spectra obtained after first derivative followed by SNV for the Benchtop (B), the handheld 1 (H1) and the handheld 2 (H2) instruments.
Figure 1: The PC2-PC3 score plot obtained with the NIR spectra after first derivative followed by SNV. NIR spectra measured on: (A) benchtop, (B) Handheld 1, (C) Handheld 2. Caption: * = C1, * = C2, * = C3, * = C4, * = C5, * = C6, * = C7, o = C8, o = C9, o = C10, o = C11, o = C12, o = C13.
Figure 4: NIR spectra obtained after first derivative followed by SNV. NIR spectra measured on benchtop (B), Handheld 1 (H1) and Handheld 2 (H2). BH1 and BH2 are the transferred benchtop spectra towards Handheld 1 and Handheld 2 respectively.
Figure 5: Transfer of class C1 spectra from benchtop towards Handheld 1: Benchtop spectrum (black), Benchtop spectrum after linear interpolation (red), Benchtop spectrum after linear interpolation and standardization (blue), Handheld 1 spectrum (green).
Figure 6: The PC2-PC3 score plot obtained with the benchtop NIR spectra after first derivative followed by SNV and transferred towards: (A) Handheld 1 and (B) Handheld 2. Caption: * = C1, * = C2, * = C3, * = C4, * = C5, * = C6, * = C7, o = C8, o = C9, o = C10, o = C11, o = C12, o = C13.
Table 1: Characteristics of the studied products. (*) Transparent (T) or opaque (O) blister. (***) ratio API and tablet weight (%).

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Manufacturers</th>
<th>Dosage (mg)</th>
<th>Blister*</th>
<th>Ratio (%) **</th>
<th>Galenic form</th>
<th>Batches</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>1</td>
<td>1000</td>
<td>T</td>
<td>91.6</td>
<td>Coated tablets</td>
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<td>500</td>
<td>T</td>
<td>86.1</td>
<td>Tablets</td>
<td>2</td>
</tr>
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<td>1000</td>
<td>T</td>
<td>86.7</td>
<td>Coated tablets</td>
<td>2</td>
</tr>
<tr>
<td>C4</td>
<td>2</td>
<td>500</td>
<td>T</td>
<td>86.1</td>
<td>Coated tablets</td>
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</tr>
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<td>1000</td>
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</tr>
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<td>500</td>
<td>T</td>
<td>84.0</td>
<td>Tablets</td>
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</tr>
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<td>500</td>
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<td>83.1</td>
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<td>500</td>
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</tr>
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<td>665</td>
<td>O</td>
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<td>250</td>
<td>T</td>
<td>36.4</td>
<td>Coated tablets</td>
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</tr>
<tr>
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<td>O</td>
<td>73.1</td>
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</tr>
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<td>67.3</td>
<td>Coated tablets</td>
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Table 2: SIMCA classification model for benchtop (B), Handheld 1 (H1) and Handheld 2 (H2) instruments. The performance of the model was evaluated using the following statistical parameters: sensitivity (Se), specificity (Sp), accuracy and error rate.

<table>
<thead>
<tr>
<th>Instruments</th>
<th>Benchtop</th>
<th>Handheld 1</th>
<th>Handheld 2</th>
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<tr>
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<td>Internal</td>
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<tr>
<td></td>
<td>Spectra</td>
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<tr>
<td></td>
<td>PCs</td>
<td>Se</td>
<td>Sp</td>
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</tr>
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<td>1,000</td>
</tr>
<tr>
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<td>1,000</td>
</tr>
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<td>1,000</td>
</tr>
<tr>
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<td>1</td>
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<td>1,000</td>
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<tr>
<td>C7</td>
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<td>1,000</td>
</tr>
<tr>
<td>C8</td>
<td>4</td>
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<td>1,000</td>
</tr>
<tr>
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<td>4</td>
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<td>1,000</td>
</tr>
<tr>
<td>C10</td>
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</tr>
<tr>
<td>C13</td>
<td>1</td>
<td>1,000</td>
<td>1,000</td>
</tr>
</tbody>
</table>

Error rate: 0,000 0,007 0,002 0,017 0,002 0,060
 Accuracy (%): 100 99,2 99,7 97,7 99,7 96,2
Table 2: SIMCA models using transferred benchtop spectra towards the Handheld 1 device. The performance of the model was evaluated using the following statistical parameters: sensitivity (Se), specificity (Sp), accuracy and error rate.

<table>
<thead>
<tr>
<th>M1 transfer</th>
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<th>M2</th>
<th>M3</th>
<th>M4</th>
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<td>External</td>
<td>Internal</td>
<td>External</td>
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<td>650</td>
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<tr>
<td>PCs</td>
<td>Se</td>
<td>Sp</td>
<td>Se</td>
<td>Sp</td>
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<tr>
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<tr>
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</tr>
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<td>1,000</td>
</tr>
<tr>
<td>C11</td>
<td>2</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>C12</td>
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<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>C13</td>
<td>2</td>
<td>1,000</td>
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</tr>
<tr>
<td>Error rate</td>
<td>0,004</td>
<td>0,020</td>
<td>0,011</td>
<td>0,020</td>
</tr>
<tr>
<td>Accuracy (%)</td>
<td>99,6</td>
<td>97,7</td>
<td>98,9</td>
<td>97,7</td>
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</tbody>
</table>
Table 3: SIMCA models using transferred benchtop spectra towards the handheld 2 device. 
The performance of the model was evaluated using the following statistical parameters: 
sensitivity (Se), specificity (Sp), accuracy and error rate.

<table>
<thead>
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<th>M3</th>
<th>M4</th>
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<td>Internal</td>
<td>External</td>
<td>Internal</td>
<td>External</td>
</tr>
<tr>
<td>Validations</td>
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<td>130</td>
<td>650</td>
<td>130</td>
</tr>
<tr>
<td>Spectra</td>
<td>PCs</td>
<td>Se</td>
<td>Sp</td>
<td>Se</td>
</tr>
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<td>C10</td>
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</tr>
<tr>
<td>C11</td>
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</tr>
<tr>
<td>C12</td>
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</tr>
<tr>
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<td>1,000</td>
<td>1,000</td>
</tr>
</tbody>
</table>

| Error rate | 0.000 | 0.038 | 0.013 | 0.052 | 0.003 | 0.013 | 0.004 | 0.013 |
| Accuracy (%)| 100  | 96.2  | 98.8  | 97.7  | 99.7  | 97.7  | 95.7  | 97.7  |