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Assessing the transfer of quantitative NIR calibrations from a spectrometer to another one

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Abstract. Many factors account for the optical differences between two near-infrared (NIR) spectrometers of the same make and type. The use of two NIR spectrometers may involve different calibrations, that can be transferred only with additional work, like bias adjustments, other sample measurements, model recalculations, conversion of spectral data, etc. In most cases direct transfers of the applications are not possible. In this study the feasibility of a calibration transfer between FOSS/NIRSystems NIR spectrometers was checked. The set of multivariate calibrations for a non-invasive identity testing and a direct determination of the water content in ceftriaxone disodium salt sterile (Rocephin®) and in a preparation containing tenoxicam active substance (Tilcotil®) filled in vials, was transferred from one spectrometer to another. Separate bias adjustments were calculated for each calibration to fit the spectral data collected on both instruments. These considerations define a practicable procedure to transfer quantitative NIR applications with a reasonable amount of additional work.

Key-words. NIR spectroscopy – quantitative calibration transfer – water content determination – multivariate calibration – bias adjustment – monochromator.

Introduction

NIR spectroscopy gradually supersedes or completes well-established analytical techniques [1,2,3,4]. Analysis by NIR spectroscopy requires elaborate multivariate calibration models, computed e.g. by principal component analysis (PCA) for identity testing or partial least squares (PLS) to quantify selected parameters. A large set of spectral and analytical data is required [5,6]. These data may be obtained from conditioned or treated samples, which are costly and difficult to prepare. It may happen that multivariate calibration models are no longer valid, e.g. in the case of instrumental alterations, drifts in optical parts, instrument servicing, or simply when the spectrometer is replaced by a similar one. Portability between different NIR equipments would avoid the duplication of calibration effort and allow the transfer of any NIR application between spectrometers. Global NIR spectrum standardization would permit the exchange of spectral data between equipments of various makes and types. This objective is not likely to be achieved in the short term. The possibility of transferring data - and calibrations - from one equipment to another one of the same make and type may be considered as a basic requirement. Unfortunately this was not a major issue for the leading NIR spectrometer suppliers. Many attempts and problem-specific solutions to calibration transfer have been proposed [7–12]. Three practical points support a NIR model transfer. Firstly, no change in prediction quality of already existing NIR calibrations should occur after the exchange of optical parts or maintenance work on a given instrument. Secondly, NIR calibrations should easily be transferred to another – equivalent – instrument in the event of a breakdown. At least, it should be possible to run already existing valid NIR calibrations on any newer instrument (forward compatibility). At the outset, NIR model development is affected by the samples available. Following factors may directly be linked to the samples and were taken into account while developing the calibrations: sample presentation over light beam, dirty sample surface when measuring through vial bottom, variations in particle shape or surface texture, variations in particle size distribution, variations in sample compression, non-homogeneous samples, dirty instrument window, experimental error in reference method, temperature, and moisture. Life cycle and availability of the NIR models, which are very sensitive to many instrumental factors are also important to routine work [13–15]. Differences in wavelength precision, linearity, and bandwidth across the whole NIR range make instruments optically different. This also concerns spectrometers, which have been modified or serviced. Following instrumental factors are of real significance and may produce a failure: possible wavelength errors, changes in signal intensity due to optical pathlength differences, internal temperature variations, effect of lamp ageing, instrument setup, differences in ceramic reference, linearity problems, ageing of the detectors, etc.

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Experimental

To assess the practicability of the transfer of selected NIR models to another instrument, a high degree of reliability in identification and quantification of currently existing NIR models must be maintained without complex model adjustments. The methodology described in this study can also be followed when new working conditions have been laid down, *i.e.* after replacing an optical component, or changing the lamp. For this purpose two spectrometers NIRSystems 5000 (instruments A and B) equipped with the Rapid Content Sampler manufactured by FOSS/NIRSystems were used to record NIR spectra at 4 nm resolution over the full NIR range from 1100 to 2500 nm. The spectrometers were located in the same laboratory and were used under the same environmental conditions. The NIRSystems 5000 spectrometer is a dispersive scanning instrument of the grating type. The measurements were made with the horizontal sample desk in the diffuse reflectance mode. Optics included a tungsten-halogen source lamp, a single monochromator with a holographic diffraction grating, and 6 uncooled lead sulfide detectors. These were distributed circularly at the base of a glass window fitted with an iris for centering. Each sample was placed on this window in the horizontal sample desk and centred over the light beam. The radiated light penetrated through the base of the sample into the substance, was absorbed, diffusely reflected, and collected. The samples were measured 3 times in replicate. A complete spectrum calculated as the mean of 32 full range scans which took approx. 40 s, made allowance for instrument variations. The apparent density of the samples was modified between two measurements by tapping the sample vial. The NIR spectra were improved by performing a mathematical pretreatment on the data. The second derivative of the spectra was used for model calculation to reduce baseline shifts and improve peak shape and resolution.

NIR calibrations were calculated and validated for direct identification of the material and determination of the water content in sealed vials of two injectable drugs produced by Roche at the Basel site. The first application was concerned with ceftriaxone disodium salt sterile (Rocephin®) filled in 15 ml septum vials, a long-acting broad spectrum cephalosporin for parenteral use. The water contents of every batch of ceftriaxone disodium salt sterile bulk substance and of filled Rocephin® vials have hitherto been measured by the Karl Fischer titration (KFT) method with double determination. This involved the preparation of samples. Aggressive chemicals had to be used and subsequently disposed of. Sample preparation required the bulk substance to be accurately weighed and the vials to be opened in advance. NIR spectroscopy was suggested as an alternative analytical method with the corresponding chemometric models to determine water contents directly in the sealed vials, thus, avoiding the preparation of samples or the use of reagents. The second application was concerned with the lyophilized formulation of tenoxicam (Tilcotil® 2 ml = 20 mg Type C) filled in 2 ml vials, an antirheumatic, antiinflammatory, and analgesic agent. The accuracy and precision of NIR determinations compared with that of the validated KFT method. The underlying NIR calibrations were unique and developed on spectrometer A.

Results

The calibration results for Rocephin® and Tilcotil® are given for the information of readers. Method development is summarized and will not be discussed in the present work. All the calibrations and corresponding NIR applications comply with the GMP standards for analytical method development currently used at the Roche laboratories. These NIR applications undergo regular controls in regard of stability and ability to reduce the impact of influencing factors over the long term.

Calibration for Rocephin®

The knowledge base for the multivariate calibration consisted of 80 samples going back to the 1994–1995 period. A separate data set known as the training base with 46 samples was used to validate the calibration. An amount of ca. 1 g of ceftriaxone disodium salt sterile was filled into empty 15 ml vials which were immediately sealed. Rocephin® vials were used directly. To extend the linearity of the model beyond the limits of the 8.0 to 10.0 % registered range, additional samples containing 5.3 to 12.8 % of water were prepared. The water contents in the knowledge base were distributed normally around the mean: range in % water 5.34 to 12.77, mean 9.00. The water contents in the training base lay within the registered range. To perform the measurements, the operator had to ensure that the base of the vials was evenly covered with powder and the lower surface clean. The complete NIR range from 1100 to 2500 nm was scanned and used. The crucial bands were the two water bands at 1450 and 1940 nm. The water contents of the selected sample vials were determined according to KFT method. The PLS calibration was calculated by using the quantitative modelling part of the commercially available NSAS/IQ^2 software from FOSS/NIRSystems. The PLS procedure combined factors (principal components) to identify the variability in the spectral data involving simultaneous correlation with the corresponding water contents. An internal cross-validation (in which the knowledge base was broken down into segments) was performed. As a rule, each segment was iteratively withdrawn while the remainder was used to calculate the model. A satisfactory model was obtained by using 6 PLS components (Fig. 1, correlation coefficient 0.994, standard calibration error 0.090, slope 1.000 ± 0.007, intercept 0.000 ± 0.006). The NIR method is unbiased with respect to the reference method. In accordance with procedures defined by the Roche pharmaceutical quality control, selected acceptance tests had to be performed. The water content of one Rocephin® vial was determined six times in succession by NIR. The resulting relative standard deviation (S. dev.) of 0.20 % showed very good repeatability on one sample. The water contents of 10 Rocephin® vials were measured by NIR and double determination. The

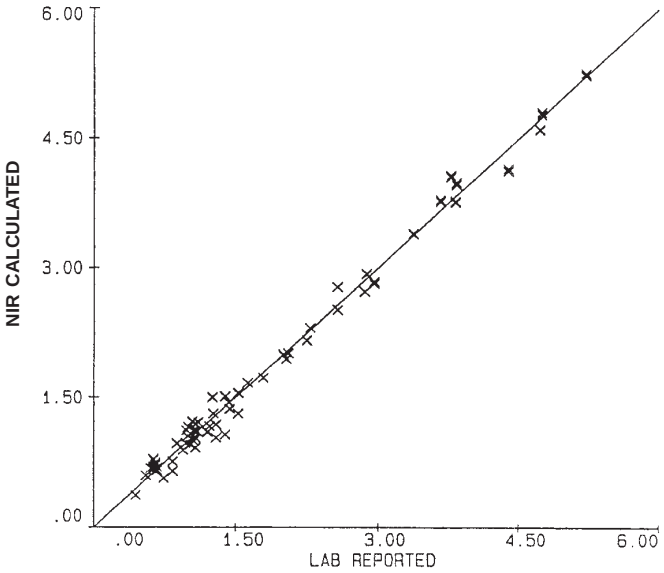

Figure 1. Calibration line for Rocephin® water content.

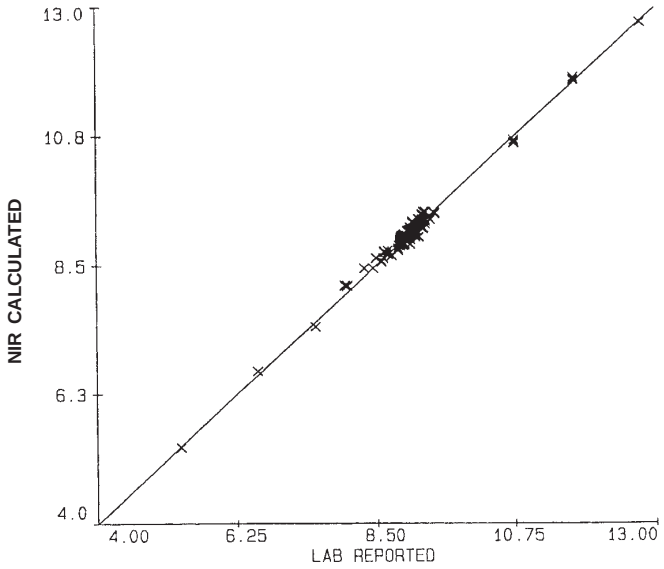

Figure 2. Calibration line for Tilcotil® water content.

resulting relative S. dev. of 0.49 % showed very good repeatability on several samples. The absolute error on ceftriaxone disodium resulting from KFT was estimated at ± 0.1 %, though ± 0.2 % was usual in routine operation. Interfering factors included ambient air humidity, speed at which the sample had been prepared, hygroscopicity of the sample. 95 % of the absolute difference between NIR and KFT determinations lay within ± 0.1 %. To confirm the efficiency of the spectroscopic method, NIR and KFT water determinations of the 46 samples of ceftriaxone disodium substance or Rocephin® vials from the training base were compared for accuracy and linearity. No significant difference was found between the two methods.

Calibration for Tilcotil®

The knowledge base for multivariate calibration consisted of 62 samples going back to the 1992–1995 period. A separate training base of another 41 samples and covering the same range as the knowledge base was used to validate the model. The linearity was extended beyond the registered range of water contents (up to 2.0 %). To have values below 1 % and substantially above 2 %, Tilcotil® samples with controlled water contents were prepared in the laboratory. The range in the knowledge base was 0.45 to 5.23 % and 0.44 to 4.76 % in the training base. The recorded NIR spectra were combined with the water contents of the corresponding samples that were determined by KFT. A PLS calibration was calculated by using the NIR spectra up to 2250 nm. The training base was directly used as an external validation set during the PLS procedure. A satisfactory model was calculated with 4 PLS components (Fig. 2, correlation coefficient 0.996, standard error of calibration 0.115, slope 1.000 ± 0.007, intercept 0.000 ± 0.008). NIR is unbiased with respect to KFT. The water content of one vial was determined 10 times in succession by NIR. The resulting relative S. dev. of 0.30 % showed very good repeatability on one sample. The water contents of 10 vials were measured by NIR and triple determination. The resulting relative S. dev. of 0.23 % showed very good repeatability on several samples. The NIR and KFT water determinations of the 41 samples from the training base were tested for accuracy and linearity and no significant difference was found between the two methods.

Calibration transfer

40 vials of Rocephin® and 33 vials of Tilcotil® were measured on different days with spectrometers A and B and stored in separate files. The water contents were predicted on the basis of spectral data recorded with both spectrometers by using the above-described calibrations for water determination. Table Ia shows the results for Rocephin®. A systematic difference was observed for predicted Rocephin® water contents between instruments A and B, with a mean value of 0.46 and a S. dev. of 0.02. It is worthy of note that the systematic error between instruments A and B for Tilcotil®, had a mean value of 0.36 and a S. dev. of 0.02. These differences were product-specific. They were also related to the prediction of water contents from spectral data recorded on instrument B. The offsets observed were stable and recalibrations were not necessary as the predicted water contents still correlated. The original calibration equations had to be modified to remove the source of error. Adjustments of the calibrations were calculated by using the percent prediction and bias adjustment programme of the NSAS/IQ^2 software. Bias adjustment was applied to each original calibration to bring the predicted values from

Table I. Calibrations on instruments A and B using two disjoint sets of 40 vials containing ceftriaxone disodium salt sterile (Rocephin®), for identification and water content determination via NIR spectroscopy. Water contents are given for A and B, with the difference A-B.
Ia – Data for prediction comparison (the original calibration was used on instruments A and B).
Ib – Prediction with bias adjustment (the original calibration used on instrument A and the adjusted calibration on B).

Ia				*Ib*			
Batch	*Instrument*		*Difference*	*Batch*	*Instrument*		*Difference*
N°	*A*	*B*	*A - B*	*N°*	*A*	*B*	*A - B*
1	8.93	8.47	0.46	41	8.76	8.76	0.00
2	8.55	8.08	0.47	42	8.83	8.83	0.01
3	8.84	8.37	0.47	43	8.92	8.92	0.00
4	8.65	8.20	0.45	44	8.73	8.73	0.00
5	8.80	8.35	0.45	45	8.83	8.85	–0.02
6	8.92	8.48	0.44	46	8.94	8.96	–0.02
7	8.95	8.48	0.47	47	8.81	8.80	0.01
8	8.94	8.47	0.47	48	8.87	8.85	0.02
9	8.82	8.36	0.46	49	8.98	8.96	0.02
10	8.94	8.48	0.46	50	8.87	8.85	0.02
11	9.09	8.61	0.48	51	9.00	9.01	–0.01
12	8.61	8.13	0.48	52	9.01	9.02	–0.01
13	8.84	8.36	0.48	53	9.05	9.04	0.01
14	8.70	8.21	0.49	54	9.12	9.11	0.01
15	9.17	8.69	0.48	55	8.65	8.62	0.03
16	9.11	8.70	0.41	56	8.76	8.79	–0.03
17	8.86	8.43	0.43	57	9.11	9.16	–0.05
18	9.15	8.72	0.43	58	9.13	9.16	–0.03
19	8.94	8.50	0.44	59	9.12	9.16	–0.04
20	8.67	8.23	0.44	60	8.86	8.89	–0.03
21	9.31	8.83	0.48	61	9.26	9.29	–0.03
22	9.35	8.90	0.45	62	9.27	9.28	–0.01
23	9.22	8.76	0.46	63	9.17	9.16	0.01
24	9.22	8.74	0.48	64	9.29	9.29	0.00
25	9.01	8.57	0.44	65	9.25	9.26	–0.01
26	9.05	8.60	0.45	66	8.86	8.89	–0.03
27	9.05	8.58	0.47	67	8.80	8.79	0.01
28	9.04	8.56	0.48	68	8.98	8.99	–0.01
29	8.35	7.87	0.48	69	9.04	9.04	0.00
30	8.78	8.34	0.44	70	8.87	8.88	–0.01
31	9.06	8.61	0.45	71	9.13	9.14	–0.01
32	8.78	8.34	0.44	72	8.94	8.94	0.00
33	8.99	8.52	0.47	73	8.72	8.71	0.02
34	8.90	8.43	0.47	74	9.07	9.06	0.01
35	9.15	8.71	0.44	75	9.01	9.01	0.00
36	8.69	8.23	0.46	76	8.70	8.70	0.00
37	9.01	8.54	0.47	77	8.88	8.89	–0.01
38	9.05	8.57	0.48	78	8.85	8.86	–0.01
39	9.16	8.68	0.48	79	8.96	8.94	0.02
40	8.75	8.28	0.47	80	8.91	8.92	–0.01
		Mean:	0.46			Mean:	0.00
		S.dev.:	0.02			S.dev.:	0.02

instrument B in line with those from instrument A. For a given calibration the bias was calculated as the difference between the averages of the values from instruments A and B, respectively. This value was, then, fixed and the resulting adjusted calibration equations were stored separately and applied to instrument B. The result adjustments were verified by comparing the water contents of additional samples measured with both instruments. 40 Rocephin® vials and 33 Tilcotil® vials were measured on different days with spectrometers A and B and stored in separate files. Predictions were carried out by using the original calibrations for instrument A and the adjusted ones for instrument B. Table Ib shows the results for Rocephin®. No systematic differences in predicted water contents were

observed between instruments A and B (mean: 0.00, S.dev.: 0.02). For brevity's sake, the results for Tilcotil® are not included but they reveal identical quantitative characteristics (mean: 0.00, S. dev.: 0.02).

Discussion

The Roche laboratories at the Basel site started using in 1995 the NIR spectroscopic determination of the water contents in Rocephin® and Tilcotil® for the monitoring of pharmaceutical quality to achieve an accuracy comparing with that of the hitherto performed KFT. The corresponding NIR analytical applications did not require sample preparation nor involve any reagent to determine the water content directly in sealed vials. It can be concluded from the above experiments that the optical differences between spectrometers A and B were offset by a bias adjustment on the calibrations for Rocephin® and Tilcotil®. A direct transfer was not possible and would result in systematic error. The method described above reasonably permits the transfer of any multivariate calibration for quantification between spectrometers of the type NIRSystems 5000 equipped with the Rapid Content Sampler. For each multivariate calibration, a sufficient number of samples had to be kept in store. This is a major drawback of the method since it involves exploration of the corresponding bias for each calibration. In case of a transfer, the samples must be measured on both spectrometers to calculate and validate the bias. Instruments may be considered as equivalent if no systematic difference is apparent between the two instruments for all NIR models after bias adjustment at the time of transfer. Once the models are adjusted, the results obtained with a second spectrometer are as accurate and reliable as those recorded with the original equipment used for model calibration. Validity of the models on the different spectrometers has to be verified on the long term independently and on a regular basis as required for any calibration.

Conclusion

Different transfer situations were examined for pharmaceutical control at the Roche laboratories with different types and makes of NIR spectrometers. In case of transfer of NIR applications between similar instruments of the same make [16,17], direct transfer is not always successful. Transfer by bias adjustment, as described in this study, can be applied for quantitative applications, which requires at least an accurate and stable wavelength-axis by construction, combined with bias adjustments that are dependent on the calibration [18,19]. Transfer by instrument standardization was also suggested. It was specific to measurements by reflectance of solids and required the regular calibration of the optical response of each instrument that involved the use of costly and sensitive NIST-reflectance standards. Systematic remeasurements of samples contained in the original calibration set, combined with a complete recalculation and validation of the NIR applications added to the complexity. The transfer of NIR applications between instruments of different makes and types was a more radical step. Direct transfer was not possible and a prerequisite was the standardization of all instruments. The conversion and edition of the spectral data sets, the new design and recalculation of all NIR applications were also required. Such a transfer was time-consuming and typically a one-way "master to slave" procedure which was not suitable for routine. In addition to lower deviations between instruments, standardized and validated methods for transfer should be proposed directly by the manufacturers and apply to reflectance or transmission models, for solids and liquids. The direct transfer of any NIR application between equipments of the same type and make, without additional sample measurements or model calculations, is obviously a minimum requirement in comparison with the ensuing benefits.

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