Increased productivity

Figure 1: DRS-8010ASC for 24 samples with the diffuse reflection unit based on elliptical mirrors in the 'praying mantis' configuration

NIR-FTIR spectroscopy has, in recent years, gained much interest due to its simple maintenance, short measuring times, high signal-to-noise ratios and excellent automation potential. With NIR-FTIR it is possible to turn time- and material consuming routine tasks into highly productive analytical processes. The largest role for NIR-FTIR is reserved for monitoring pre-defined criteria in quality control. In particular, researchers in the pharmaceutical industry are increasingly using the potential which NIR spectroscopy has to offer.

A time-consuming sample preparation procedure is usually not necessary. The acquired NIR spectra generally exhibit broad bands that can rarely be individually assigned. A particular sample is therefore better characterised by its entire spectrum. The required selectivity can be attained with the use of calibration models. Via suitable models, even (NIR transparent) packaged samples can be analysed right through the packaging material. This option is frequently used in the pharmaceutical industry.

In order to generate a calibration model, standards are needed which reflect the expected variance in the samples to be analysed. In addition to sample composition and particle size, the annually varying composition of biological products must be taken into account. The acquired NIR spectra of the standards are subsequently analysed using chemometric methods.

Chemometrics

The strongly overlapping absorption bands in the NIR range of the spectrum necessitate a multivariate mathematical procedure in order to fully use the advantages of the large signal to noise ratio despite the overlapping bands. In a univariate procedure, a correlation between a characteristic and only one measuring quantity is established, whereas in a multivariate procedure several measuring quantities are used to describe a characteristic. The selection of suitable wavelengths (for instance **M**ulti **L**inear **R**egression, MLR method) is, however, not trivial with respect to overlapping bands, interferences and matrix effects and requires an extensive optimisation procedure. Alternatives to these wavelength-selective methods are factor-analysis procedures such as PLS (**P**artial **L**east **S**quare) and PCR (**P**rincipal **C**omponent **R**egression), which are based on analyses of the entire spectra. PLS combines the fundamental characteristics of multivariate methods such as CLS (**C**lassical **L**east **S**quare) and ILS (**I**nverse **L**east **S**quare). The PLS method reduces the very large amount of spectral data, whereby the spectrum is reduced into suitable factors that describe the spectrum.

The fundamental idea of the PLS method is to obtain as much (concentration-) information as possible from the spectra.

The Partial Least Square

The Partial Least Square (PLS) method is one of the most frequently used chemometric tools to extract concentration-information from acquired spectra. PLS is an effective method for quantitative analysis of complex spectra

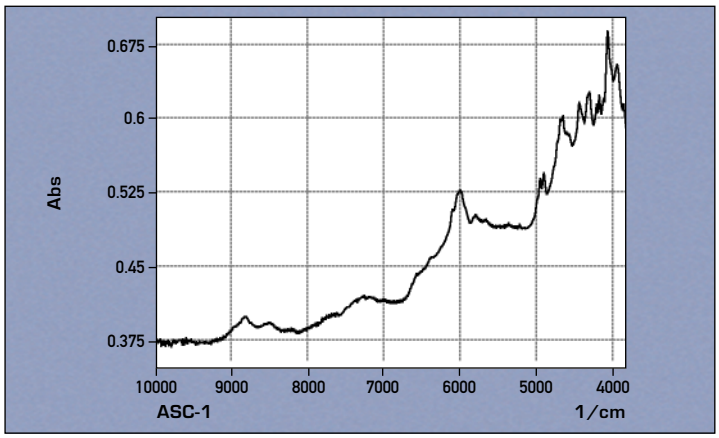

Figure 2: NIR spectrum of a powder sample consisting of 39.97 % aspirin, 4.95 % caffeine and 55.08 % paracetamol, measured with the diffuse reflection technique

with NIR-FTIR technology

with overlapping absorption bands and is especially suitable when a large number of standard spectra (reference spectra) are available with very little information on the absorption behaviour of the compounds. The PLS method is based on the principle of using as few latent variables (non-observable variables) as possible in the interpretation of the entire spectrum (full spectrum analysis).

In other words, there is no separate regression step. PLS carries out the reduction of spectral data and the information on concentration simultaneously. A further difference with procedures such as MLR or CLS is that both methods use the spectrum directly whereas PLS first reduces the spectrum in so-called 'Score'- and 'Loading' vectors.

Internal and external validation

During an internal validation, one aliquot sample from the standard samples is used to test the validation. When an external sample (not a standard sample) of known concentration is used, this is considered as external validation. Shimadzu's IRsolution software uses the internal validation procedure. During internal validation, the sample spectra are extracted one by one and the difference between the real and the predicted concentration is squared. This operation is carried out for all samples used in the calibration and the PRESS value (Prediction Residual Sum of Squares) is obtained as the sum of squares of the differences.

Furthermore, the number of components that describe the system is varied until the corresponding PRESS calculations reach a minimal value. This method is referred to as 'Leave-One-Out'. In the IRsolution software two versions of the PLS algorithm (PLS-I and PLS-II) are available. Although the difference between these two methods is minor, it leads to important differences in the results.

- PLS-I: A separate set of 'Score'- and 'Loading' vectors is calculated for each system component. This set of vectors is optimised for all individual components.
- PLS-II: The 'Score'- and 'Loading' vectors are calculated for all components simultaneously. The vectors are not optimised separately for individual components.

This is why the PLS-I method generally delivers the more accurate value. However, some cases have been described in literature where PLS-II as well as PCR delivers better results. Unfortunately, there are no generally applicable rules whereby one can chose the most suitable procedure. Experience and perseverance when trying different methods is, therefore, still essential.

Report of PLS Calibration Calibration Table			
Algorithm	PLS-I		
Number of components	3		
Number of references	15		
Range	3799.65 - 10002.52 cm^{-1}		
Preprocessor	PLS Calibration		
Centered data	Yes		
Component	Aspirin	Caffeine	Paracetamol
Number of factors	4	4	4
Correlation coeff.	0.99832	0.99813	0.99850
MSEP*	0.00314	0.00348	0.00279
SEP **	0.05601	0.05899	0.05286
X Leverage warnings	0	0	0
Y Residual warnings	0	0	0

Table 1: Excerpt from the calibration report *MSEP: Mean Square of Prediction **SEP: Standard Error of Prediction

Three-component system: Aspirin – Caffeine – Paracetamol

A classic three-component system was analysed using Shimadzu's IRPrestige-21 FTIR spectrometer with NIR accessory and the DRS-8010ASC diffusion reflection unit with autosampler (24 samples). All mathematical calibrations were carried out using the IRsolution software version 1.10.

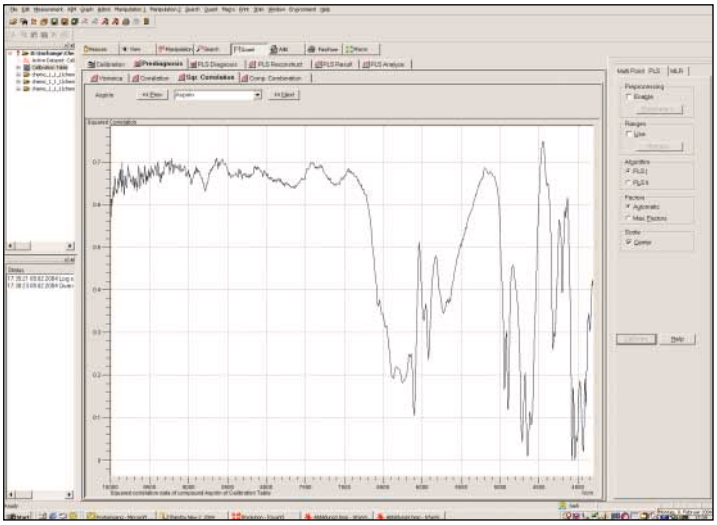

Figure 3: 'Square Correlation' spectrum for aspirin

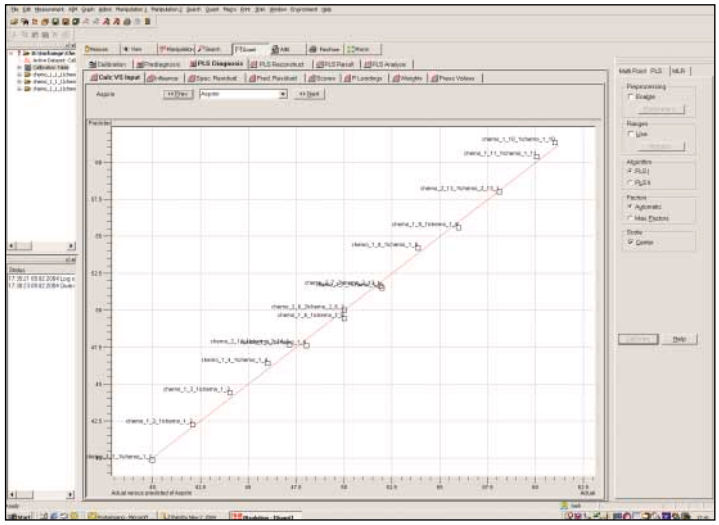

Figure 4: graphical representation of the predicted (*predicted*) versus the measured concentrations (*actual*) for aspirin

A total of 15 powder samples of different concentrations were used for the calibration in concentration ranges of: aspirin (acetyl salicylic acid) 40 % - 60 %, caffeine (1,3,7-trimethylxanthine) 4.95 % - 25.95 % and paracetamol (4-acetylamidophenol) 18.90 % - 55 %. In order to obtain optimal conditions (equal particle sizes) for diffuse reflection, all samples were ground to a fine powder and mixed. The resulting powder was placed in small aluminium holders (Al pellet holder, diameter 6 mm) and lightly compacted with a pestle in order to obtain a smooth surface. Figure 2 shows a typical NIR spectrum that was obtained using this method.

PLS-I calibration

In order to improve the PLS-I calibration, all spectra were first centred using the IRsolution software. For this purpose, the average value spectra, obtained from all calibration spectra, were subtracted from individual spectra. It is commonly recommended in daily laboratory practice, to carry out a baseline correction in order to correct for possible drift of the baseline. Table 1 shows an excerpt of the PLS calibration report.

As can be concluded from Table 1, all three compounds (aspirin, caffeine, paracetamol) can be determined with a correlation coefficient of $r > 0.998$ and a standard error of prediction of $SEP < 0.06$. Here the excellent suitability of the PLS method for multi-component systems is evident.

Based on the diagnosis results of the IRsolution software, it is possible to make more detailed deductions from the NIR spectra. For example, under the title Variance, Correlation vs. Sqr. Correlation, information on the various spectral ranges and their significance for the correlation can be obtained. Figure 3 shows the obtained square correlation spectrum for aspirin.

The square correlation graph shows which ranges of the NIR spectra correlate with the concentration of the three compounds. The correlation values lie between +1 and –1. As can be easily seen, the wavelength range between 7000 and 3800 cm^{-1} is especially suitable for PLS factor analysis due to its strong concentration correlation. In this range the greatest variations in the spectrum arise from the dependence on concentration.

Using the option Calc vs. Input (Figure 4), a quick overview on the quality of the calibration can be obtained. The outliers are automatically marked with a red cross (this example does not show any outliers). In the graph, the measured concentrations are plotted against (depending on the selected unit) the calculated (predicted) values.

For a better overview, the IRsolution software automatically writes the data file name next to each measuring point. In addition, the software addresses the following diagnosis points:

Used composition in %			
Sample name	Aspirin	Caffeine	Paracetamol
test_sample_1_1	50.11	14.96	35.93
test_sample_1_1_1	50.11	14.96	35.93
test_sample_3_1	46.43	10.47	43.10
test_sample_7_2	51.90	17.00	31.10

Table 2: Used composition in %

- **Influence:** Influence of the individual standard spectra on the calibration
- **Spectral Residual and Predicted Residual:** Shows the deviations from the predicted concentrations, for instance expected versus the measured spectrum
- **Scores:** Score-score plots for each component. This enables the recognition of information on the compound as well as the personal style of preparation
- **P Loadings and weights:** Generates the loading, for instance 'Weight Plot' for individual factor (four factors in this example, Table 1). Based on the individual plots, the significance of the calibration can be estimated for each factor. The stronger the noise and the smaller the variance in the spectrum, the lower is its significance for the quality of the calibration. Ideally, the number of factors should correspond with the number of chemical components (aspirin, caffeine, paracetamol)
- **PRESS values:** This function shows a graphical representation of the 'Prediction Residual Error Sum of Squares' for each factor
- **PLS Reconstruct:** This function is used to analyse the error between the actual and the simulated spectrum and to be able to adjust the PLS factors
- **PLS-Analysis:** Using PLS analysis, the determination of concentrations of unknown samples can be easily carried out.

In this example, a total of four samples of known composition was used as testing criterion for the robustness of the calibration. Tables 2 and 3 summarise the concentrations used (composition in %) and the results that were obtained via the PLS-I method.

The test samples 1_1 and 1_1_1 are samples from the same powder mixture that have been placed onto two different sample holders. They deviate by less than 2 % from the used concentrations. The deviations between the samples 1_1 and 1_1_1 are also very small (> 0.1 %), due to the excellent reproducibility of Shimadzu's IRPrestige-21 system. Test sample 3_1 shows a deviation of 1.8 % for caffeine up to approximately 3.3 % for paracetamol, which is very close to the expected values. Of interest is test sample 7_2, which shows higher than average deviations of 6 % for caffeine and 24.1 % for paracetamol. For this test sample, aspirin and caffeine from a different batch than the standard was used. In spite of the same quality, very clear differences in the PLS-I analysis can be seen.

Conclusion

These few examples show very clearly the possibilities, as well as the limitations of the PLS method. For a robust (excellent prediction of the concentration) calibration, it is absolutely necessary to take into account the possible variations in the source products. Particularly in the analysis of products of biological origin, a seasonal update of the calibration procedure is recommended in order to maintain robustness.

Once this hurdle in the calibration is overcome, NIR-FTIR spectroscopy in combination with the DRS-8010ASC diffuse reflection unit offers the possibility for fast and cost effective qualitative and quantitative analysis of NIR-active compounds. Especially for powder samples, diffuse reflection enables fast analysis without the need for further sample preparation. NIR-FTIR spectroscopy also offers the possibility of non-destructive analysis of NIR transparent packaged products right through the packaging material for online quality control.

Results optained with the PLS-I method						
Sample name	Aspirin	Resid. 1	Caffeine	Resid. 2	Paracetamol	Resid. 3
test_sample_1_1	49.2041	0.0698462	14.1642	0.0698724	36.7629	0.0698625
test_sample_1_1_1	49.1671	0.0553044	14.1315	0.0553382	36.8132	0.0553206
test_sample_3_1	45.3284	0.418377	10.2883	0.418396	44.5692	0.418387
test_sample_7_2	55.237	0.206874	20.1144	0.206971	25.0618	0.206929

Table 3: Results optained with the PLS-I methode

TELEGRAM

Fully automatic and precise

New autosampler for UV-VIS and RF

Shimadzu offers, together with CETAC Technologies, two new autosamplers for UV-VIS- and RF spectrometers.

The ASX-520 and the ASX-260 are two autosamplers that distinguish themselves by high sample throughput and high precision. Despite its small size, the ASX-520 can handle up to 360 samples (180 samples in the ASX-260) and guarantees fast and simple analyses of large sample numbers even when bench space is limited. Due to its easy installation, the instrument can be build up and running fully functional within the day of delivery.

Five different sample racks can be selected, holding 90 samples (7 mL), 60 samples (14 mL, standard), 40 samples (20 mL), 24 samples (30 mL) or 21 samples (50 mL).

The autosamplers are available for the following spectrometers: UV-2401PC / 2501PC, UV-1700 Pharmaspec, UV-1650PC, UVmini-1240, RF-1501 and RF-5301PC.