# Quality control of finished pharmaceuticals by NIR-spectroscopy

**Julia Petri[a], Astrid Kaunzinger[a], Andreas Niemöller[b], Frank Stieneker[c] and Michael Karas[d]**

[a] *Central Laboratory of German Pharmacists. Carl-Mannich-Str. 20. 65760 Eschborn. Germany. J.Petri@zentrallabor.com. A.Kaunzinger@zentrallabor.com*

[b] *Bruker Optik GmbH. Rudolf Plank-Str. 23. 76275 Ettlingen. Germany. Andreas.Niemoeller@brukeroptics.de*

[c] *APV. International Association for Pharmaceutical Technology. Kurfürstenstr. 59. 55118 Mainz. Germany. stieneke@apv-mainz.de*

[d] *Departement of Instrumental analytical Chemistry, Institute of Pharmaceutical Chemistry. Johann Wolfgang Goethe-University. Marie-Curie-Str. 9-11. 60439 Frankfurt. Germany. Karas@iachem.de*

## Introduction

The Near Infrared (NIR)-spectroscopy has developed to a rapid, simple and non-destructive method for the quantification of active ingredients in tablets. Time consuming sample preparation becomes redundant, NIR methods do not generate any waste compared to other procedures like HPLC and the complete analysis of a sample with the NIR spectrometer only takes a few minutes. Because of these reasons the NIR spectroscopy is meanwhile established as a powerful tool not only in the identification of raw materials but also in the end-product testing of tablets in the pharmaceutical industry[1,2].

In this work we investigated the possibilities to set up calibration models with tablets of the same active ingredient but from different manufacturers having non-uniform sizes, forms, colours and especially different excipient matrices. For this purpose we measured nearly all tablets available on the German market of three different preparation groups. The active ingredients were the well known anti-inflammatory and analgesic drug Acetylsalicylic acid (ASS) and the two anti-hypertensive acting substances Atenolol and Enalapril Maleat.

Because in the first step of our investigations we wanted to check if such calibration models are basically feasible, we did not perform an according reference analysis but used the declared assay of the tablets, which was converted in percent of active ingredient per tablet. Only for model development of the Enalapril tablets we used values determined by HPLC.

In order to make a comparison between the transmission and the reflectance measurements, the ASS and Atenolol tablets were measured in both modes. To evaluate the possibility to get quantitative information through blister material, we used the fibre-optic probe to measure the blister packed ASS tablets and set up an according calibration model.

## Experimental

All measurements were carried out with a Bruker FT-NIR-spectrometer VECTOR 22/N (Bruker Optik GmbH, Ettlingen, Germany), equipped with a transmission unit, different tablet holders for sample presentation and a fibre-optic probe to measure the tablets in reflectance. Each spectrum was recorded with 32 scans.

For evaluation of spectra the OPUS software with QUANT 2 package and implemented optimisation option was used, calibrations were calculated by PLS. Full cross validation was performed in each case and in order to compare the results of the diverse calibrations the generated root mean square error of cross-validation (*RMSECV*) was looked at. In the final step the calibrations were validated with independent samples, which were not included in the calibration and the root mean square error of prediction (*RMSEP*) was calculated. In some cases we observed that the value of the *RMSEP* was lower than the *RMSECV*. This might be explained by the small number of samples and the fact that the *RMSEP* was highly dependent on which kind of product is left out for validation.

The reference analysis of the Enalapril tablets was performed on a Jasco HPLC system with autosampler and Borwin HSS+1500 software for data evaluation.

### Acetylsalicylic acid tablets

In our investigations most available ASS products of the German market were involved. As dosage units 75 mg, 100 mg, 400 mg and 500 mg tablets were represented, which correspond to contents of Acetylsalicylic acid per tablet from 62% to 87%. Several batches of one manufacturer were included in the calibrations. Because there were not enough tablets from each manufacturer available, the average weight was determined only out of three tablets.

In the majority of cases two tablets from each batch and manufacturer respectively were measured from each side, both in transmission and reflectance. For the blister calibrations mostly two tablets from the same lot were measured once with the fibre probe.

### Atenolol tablets

All available tablets of the German market were measured in transmission and reflectance on the spectrometer and corresponding calibrations were calculated. All tablets were of the dosage unit 100 mg, which corresponds to assays between 21% and 39%. Only one batch of every manufacturer was involved in the calibration. Every tablet was measured from both sides.

### Enalapril tablets

All available Enalapril 25 mg tablets of the German market were involved in the investigation, the assay of Enalapril Maleate ranged between 6% and 12%. In this case we used another procedure as for the ASS and Atenolol tablets. We did not use the declared assay of the pharmaceuticals, instead we carried out a HPLC reference method according to USP 26, monograph "Enalapril Maleate Tablets". As provided in this monograph, we had to use ten tablets as composite samples for the determination. For this reason we measured ten tablets both sided in transmission with the NIR spectrometer prior to analysis by HPLC. In order to set up the calibration we calculated the average spectra of the twenty measurements per manufacturer. The value of the HPLC assay determination was assigned to the according average spectrum and the calibration was calculated.

## Results

### Acetylsalicylic acid tablets

#### *Transmission and reflectance measurements*

The spectra of the ASS tablets show no significant differences in their appearance, it seems they have similar excipient matrices, which is corroborated by the respective package leaflet, where in every case corn starch and microcrystalline cellulose are listed as adjuvants. None of the tablets involved in the calibration was coated, hence the measurement could be performed both in transmission and reflectance without any problems. The results of the calibrations are represented in Figure 1 and 2 and in Tables 1 and 2.
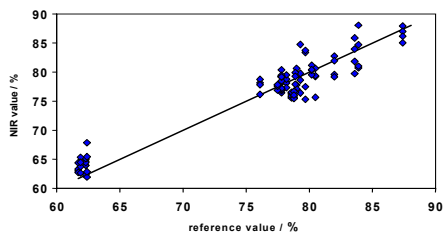
**Figure 1. Calibration plot of Acetylsalicylic acid in transmission.**

**Table 1. Results.**

| Acetylsalicylic acid tablets in transmission | | | |
|---|---|---|---|
| **Calibration** | | **Validation** | |
| No. of preparations | 14 | No. of preparations | 4 |
| Dosage range / % | 62-87 | Dosage range / % | 61-84 |
| No. of samples | 28 | No. of samples | 8 |
| No. of spectra | 106 | No. of spectra | 32 |
| No. of PLS ranks | 3 | | |
| R2 / % | 98.0 | | |
| RMSECV / % | 1.1 | RMSEP / % | 2.4 |
| Spectral range (cm⁻¹) : 12500-9500; 8752-7247 | | | |
| Data pre-treatment: vector normalisation | | | |



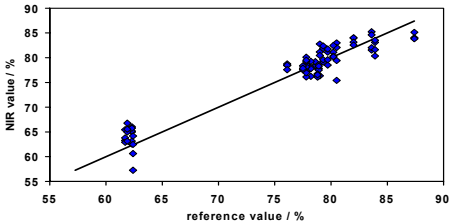**Figure 2. Calibration plot of Acetylsalicylic acid in reflectance.**

**Table 2. Results.**

| Acetylsalicylic acid tablets in reflectance | | | |
|---|---|---|---|
| **Calibration** | | **Validation** | |
| No. of preparations | 12 | No. of preparations | 4 |
| Dosage range / % | 62-87 | Dosage range / % | 62-84 |
| No. of samples | 23 | No. of samples | 6 |
| No. of spectra | 92 | No. of spectra | 25 |
| No. of PLS ranks | 5 | | |
| R2 / % | 92.5 | | |
| RMSECV / % | 2.1 | RMSEP / % | 1.7 |
| Spectral range (cm⁻¹) : 10804-7398; 5701-4848 | | | |
| Data pre-treatment: first derivation and MSC | | | |

In this case we found no significant differences between the transmission and the reflectance measurements. While the calibration of the transmission spectra shows a better coefficient of correlation, a lower *RMSECV* and only three PLS ranks, the reflectance method offers a lower *RMSEP*. As mentioned before, this may be attributed to the small amount of samples.

The reason for the similar quality of both calibrations might be on the one hand the high concentration of Acetylsalicylic acid in the tablets and on the other hand the missing reference values. In conclusion both kinds of measurements make a calibration possible, despite different manufacturers, sizes and surface conditions.

### Measurements through blister material

The tablets were packed in two different blister materials, either in polypropylene foil with a thickness of 300 μm, or in a polyvinyl chloride foil with a thickness of 250 μm.

Five PLS ranks were needed to describe the required information of the spectra and we observed a coefficient of correlation of only 85.6%, but at least a clear distinction between different dosage units is possible and a fast, easy quality control of blister packed tablets is feasible without any sample loss.
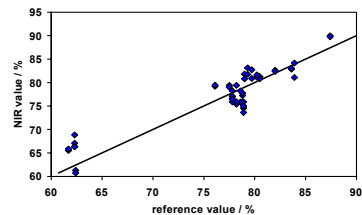


**Figure 3. Calibration plot of Acetylsalicylic acid in blister**

**Table 3. Results.**

| Acetylsalicylic acid tablets through blister | | | |
|---|---|---|---|
| **Calibration** | | **Validation** | |
| No. of preparations | 13 | No. of preparations | 4 |
| Dosage range / % | 62-87 | Dosage range / % | 61-84 |
| No. of samples | 23 | No. of samples | 6 |
| No. of spectra | 47 | No. of spectra | 13 |
| No. of PLS ranks | 5 | | |
| R2 / % | 85.6 | | |
| RMSECV / % | 2.7 | RMSEP / % | 1.8 |
| Spectral range (cm⁻¹) : 8250 - 7398 | | | |
| Data pre-treatment: MSC | | | |

## Atenolol tablets

The transmission spectra of the Atenolol are tablets shown in Figure 4. We can observe that the spectra differ clearly from each other. Some of them show a significant talc peak at about 10,500 cm$^{-1}$, others show total absorption between 8,400 cm$^{-1}$ and 8,200 cm$^{-1}$. Most of the tablets were coated, only six tablets were not encased with a film coating. With exception of two tablets they were white and from similar form and size, but the adjuvants were non-uniform. We can roughly divide them in two groups, the coated and the non-coated tablets. The coated ones can again be separated in a group with and a group without talc but



**Figure 4. Atenolol tablets in transmission**

with gelatine. The excipient matrix of the non-coated tablets was from various nature, partly the tablets contained lactose or polyvinylpyrrolidone. In general it was not possible to find two tablets with exactly the same adjuvants.
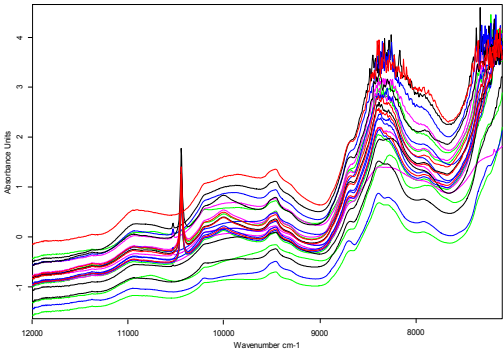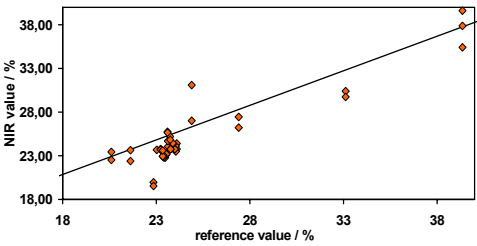


**Figure 5. Calibration plot of Atenolol in reflectance**

**Table 4. Results.**

| Atenolol tablets in reflectance | | | |
|---|---|---|---|
| **Calibration** | | **Validation** | |
| No. of preparations | 21 | No. of preparations | 3 |
| Dosage range / % | 21-39 | Dosage range / % | 24-33 |
| No. of samples | 21 | No. of samples | 3 |
| No. of spectra | 45 | No. of spectra | 6 |
| No. of PLS ranks | 5 | | |
| R2 / % | 86.5 | | |
| RMSECV / % | 2.0 | RMSEP / % | 1.2 |
| Spectral range (cm$^{-1}$) :11784-8899 | | | |
| Data pre-treatment: MSC | | | |



**Figure 6. Calibration plot of Atenolol in transmission.**

**Table 5. Results.**
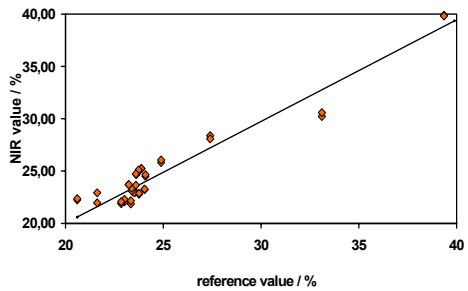
| Atenolol tablets in transmission | | | |
|---|---|---|---|
| **Calibration** | | **Validation** | |
| No. of preparations | 20 | No. of preparations | 4 |
| Dosage range / % | 21-39 | Dosage range / % | 24-33 |
| No. of samples | 20 | No. of samples | 4 |
| No. of spectra | 39 | No. of spectra | 8 |
| No. of PLS ranks | 4 | | |
| R2 / % | 93.0 | | |
| RMSECV / % | 1.1 | RMSEP / % | 0.35 |
| Spectral range (cm$^{-1}$) : 12004-11309; 10623-10276; 8895-8548 | | | |
| Data pre-treatment: vector normalisation | | | |

The figures show clearly the known advantage of transmission measurements of coated tablets compared with reflectance. In the first case we achieved a *RMSECV* of 1.1% and a correlation

coefficient of 93%, the calibration of the reflectance measurements only exhibits a *RMSECV* of 2.0% and a correlation coefficient of 86.5%. This result is not very surprising, because most of the analysed tablets were coated. Reflectance measurements of coated tablets are only appropriate if the purpose is the characterisation of the coating[3,4].

However, by using the transmission mode it was possible to develop a satisfying calibration model, although the matrix was of different nature.

### Enalapril tablets

The transmission spectra of the Enalapril tablets are shown in Figure 7. The non-coated tablets were of various nature. Round, oblong or triangular, the colours ranged from red to pink or white. Some of the tablets had very distinctive breaking notches or embossments others were totally plane. Also size and thickness varied from tablet to tablet. Surprisingly the exc

ipients were not as different as it seemed. Most tablets contained lactose, corn starch, magnesium stearate and sodium hydrogen carbonate. Some tablets contained additionally talc, povidon, crospovidon, mikrocrystalline cellulose or different kind of starches.



**Figure 7. Enalapril spectra in transmission**

As shown in Figure 8 and Table 6, we obtained very satisfying results with high correlation coefficient and low RMSECV, also the RMSEP shows with 0.25% a relatively low value. This was possible although the content of Enalapril Maleate reached only from 6% to 12%. In this case it becomes apparent, that reference values are of great advantage to reach well calibration results, especially if we compare the results to our accomplished calibrations with the two other pharmaceutical products.
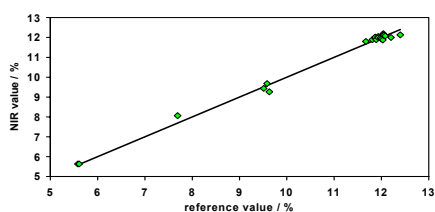


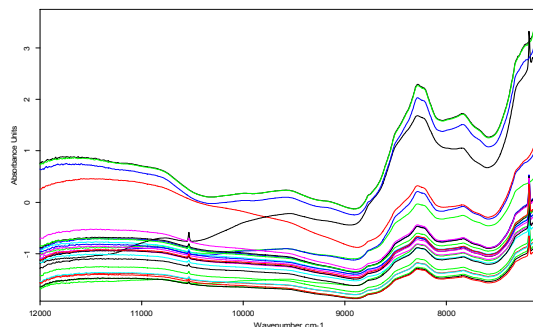| Enalapril tablets in transmission | | | |
|---|---|---|---|
| **Calibration** | | **Validation** | |
| No. of preparations | 22 | No. of preparations | 4 |
| Dosage range / % | 6-12 | Dosage range / % | 6-12 |
| No. of samples | 22 | No. of samples | 4 |
| No. of spectra | 22 | No. of spectra | 4 |
| No. of PLS ranks | 5 | | |
| R2 / % | 99.4 | | |
| RMSECV / % | 0.16 | RMSEP / % | 0.25 |
| Spectral range (cm-1) : 9060-8567; 8080-7587 | | | |
| Data pre-treatment: first derivation and vector normalisation | | | |

**Figure 8. Calibration plot of Enalapril.**          **Table 6. Results.**

## Conclusion

The results in this work show clearly, that it is possible to set up calibration models of an active ingredient in tablets independent from manufacturer and therefore independent from adjuvants or optical appearance. The ASS and Atenolol calibrations should be optimised by using results of an according reference analysis to reach better calibrations like in the case of the Enalapril tablets, but of course one has to consider which purpose these methods should suite. If the aim is to control the dosage unit of an ASS tablet, then our method will be sufficient and if the weight of the tablet is

known, it would be possible to measure the tablet directly in the blister - distinctions between different dosage unit are surely feasible with the calibrations. But if the intention is a quantification of Acetylsalicylic acid or Atenolol in the tablet up to an accuracy of one or two percent, then these methods has to be optimised especially with suitable reference analysis in order to get calibrations of similar quality like the one of Enalapril.

There might be some interesting future operational areas of such methods. For instance the simple and time saving detection of counterfeits might be possible. Then it would not be necessary to set up calibrations for every particular finished pharmaceutical but just to develop methods that concentrate on the active ingredient in a tablet. Consequently it would not matter from which origin the tablet is.

Perhaps similar methods are conceivable for the application in pharmacies. German pharmacists are committed to control the quality of finished pharmaceuticals with a purely visual investigation. With NIR measurements of blistered tablets it would be possible to make these examinations much more efficient, because it would be possible to get more information about the pharmaceutical without any loss of material.

As we can see in the case of the Enalapril analysis, also pharmacopoeial methods could be replaced by NIR spectroscopy. You do not only have the opportunity to determine the assay of active ingredients in the tablets by analysing composite samples, but furthermore you can simultaneously get information about content uniformity for example.

## References

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