Calibration transfer applied for selected medicinal plants using the Shenk-Westerhaus algorithm

Hartwig Schulz and Sven Pfeffer

Federal Center for Breeding Research on Cultivated Plants, Institute of Plant Analysis, Neuer Weg 22-23, D-06484 Quedlinburg, E-Mail: H.Schulz@bafz.de

Introduction

During the last years numerous NIR spectroscopy methods have been successfully introduced as an efficient tool for rapid and reliable analysis of secondary metabolites in various medicinal plants[1-3]. Generally, the development and maintenance of these calibrations is very time-consuming; therefore it is useful to perform central calibrations and to transfer them to individual NIR instruments at different locations. The ability to transfer quantitative calibration models directly from one instrument to another one with no recalibrations or mathematical manipulations has several benefits especially in terms of reliability and analysis costs.

In the last two decades several attempts have been made to solve the problem of transferring spectra and calibrations between instruments as a consequence of different absorption intensities, resolutions and wavelength shifts. The first attempt to deal with instrumental differences was by means of bias/slope correction of predicted values[4]. An alternative approach has been to standardize different instruments with either a single standard[5,6] or a set of standardisation samples representing the spectral properties of the material under study[7]. In order to keep the discrepancies between spectrometers as low as possible, usually instruments of the same type are used in a network. However there have been also attempts to standardise completely different types of instruments by use of absorption and wavelength standards and a subsequent software resolution fit[8-10].. As an example for other medicinal plants this study demonstrates the transferability of NIR calibrations between the same spectrometer types for estimating the echinacoside content in Echinacea roots (*E. pallida* Nutt. and E. *angustifolia* DC) and the amounts of linalool, estragole and eugenol in the essential oil of various basil cultivars (*Ocimum basilicum* L.).

Sample Material and Reference Analysis

Echinacea and basil plants were cultivated in the experimental garden of the Federal Center for Breeding Research on Cultivated Plants in Quedlinburg (Germany). In order to determine the echinacoside content approx. 1 g of the powdered echinacea root was extracted with 100 mL methanol in a Soxhlet apparatus. The resulting extract was evaporated to dryness and the residue was taken up in 25 mL of 85% o-phosphoric acid/acetonitrile (1:1000 v/v). After centrifugation at 3000 rpm an aliquot of the sample was analysed by HPLC (Waters HPLC system consisting of two pumps (model W 515), an automatic liquid chromatographic injector W 717, a photo diode array detector W 996, a UV/Vis detector W 486 and a workstation with Millenium 32 software of Waters). The separation was performed using a Luna 5 µm C18 reversed-phase column (250 x 4.6 mm i.d., Phenomenex). Eluents were 85% o-phosphoric acid/water (1:1000 v/v) and 85% o-phosphoric acid/acetonitrile (1:1000 v/v); a gradient elution programme was applied according to earlier studies[11]. The column was kept at 22°C using a column oven. Flow rate was 1 mL/min; UV-detection was set at 330 nm. Different calibration samples of echinacoside were prepared in the

concentration range between 0.01 and 6 mg/100 mL methanol from a methanolic stock solution by addition of the eluent mixture.

The air-dried and crushed basil herbs were hydro-distillled and the essential oils received were analysed by GC-FID and GC-MS^3. Identification of the detected compounds was based on their relative retention times and their mass spectra in comparison with those observed by the individual pure standard substances. The percentage composition was computed from the GC peak areas according to the 100 % method without using any correction factors.

NIRS Measurements and Chemometrics

All measurements (1100-2500 nm) were performed on two dispersive NIR spectrometers (NIRSystems 5000, Foss Instrumets Inc., Hamburg, Germany). The powdered echinacea root samples were transferred into rectangular cups (51 x 64 mm, 11 mm depth) and analysed twice with 32 scans each time. The individual basil oil samples (approx. 600 µL) were measured in quartz cells (diameter: 4.7 cm) in transflection mode using a gold reflector with a defined path length (0.2 mm). Pretreatment of spectra, determinations of standardisation files, transfers of spectra and statistical calculations were carried out with the commercial statistic programme WINISI (Infrasoft Intern. Inc., Port Matilda, USA). All data in the calibration set were checked carefully to detect and eliminate outlier samples. The calibration transfer was performed using the Shenk-Westerhaus algorithm; in this context spectrometer 1 was defined as “master” and spectrometer 2 as “slave” instrument. All spectra measured on both spectrometers were mean-centred in order to minimize the spectral offset. Samples were measured first on the “slave” instrument and the calibration model built on the “master” was used to compute the values to be predicted applying different standardisation methods (calibration without standardisation, calibration with check cell, calibration with sample).

Results

As presented in table 1 the quality of the individual PLS calibrations obtained with both Foss spectrometer systems show very good agreement regarding the NIRS statistics. As to be seen in figure 1 best results for prediction of the echinacoside content were obtained performing a calibration transfer without standardisation, whereas a standardisation with the check cell resulted in predictions differing considerably from those of the master calibration.

Contrary to that, the calibration transfer for estragole content in essential oil of basil shows only small differences regarding the four applied standardisation methods (figure 2). Generally, the results exemplarily reported here show that the applied cloning technique of Shenk and Westerhaus was found to be suitable to create reliable transfer equations for the selected NIRS calibrations. The described procedure gave results comparable to those achieved by individual calibrations on each instrument. Unfortunately, a calibration transfer to spectrometers of other manufacturers resulted in relatively high prediction errors. Therefore, in order to guarantee a reliable calibration transfer within a NIRS network, it is recommended to use comparable instruments for that purpose.

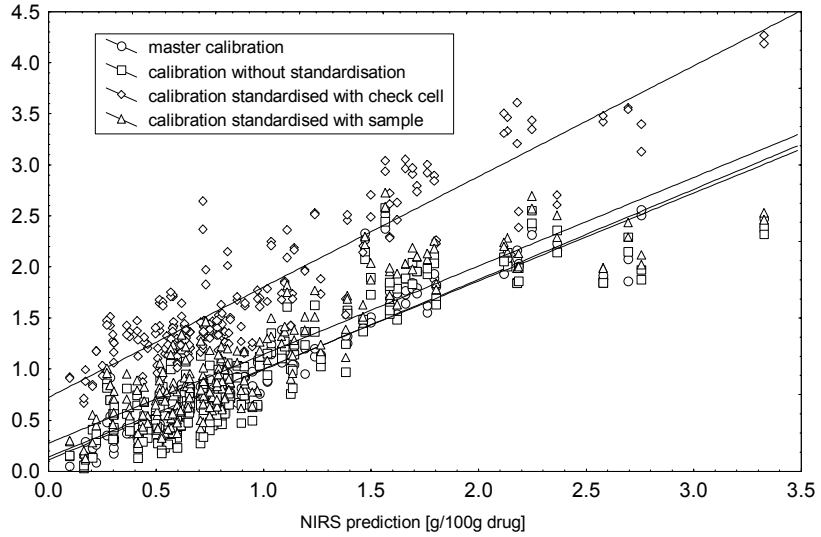

Figure 1. Results of the calibration transfer between two Foss instruments for the echinacoside content in *Echinacea* roots based on mean-centred spectra.

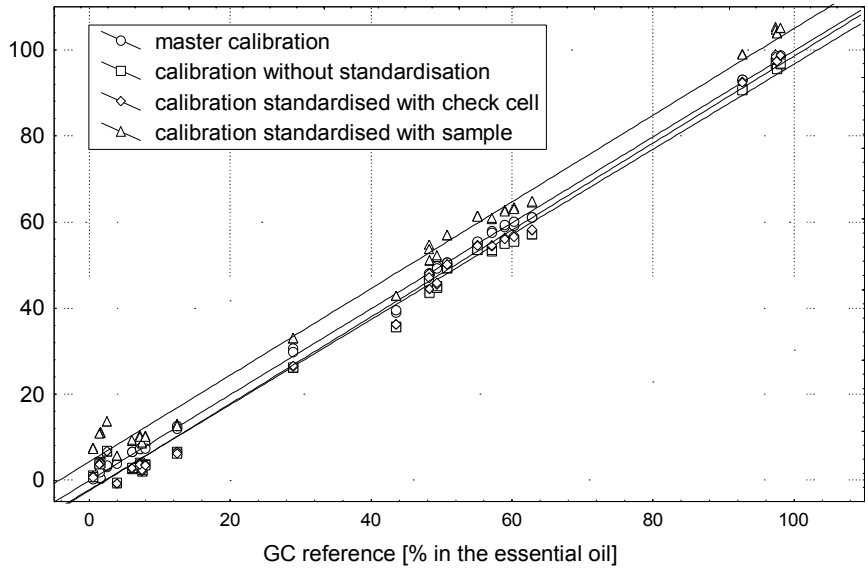

Figure 2. Results of the calibration transfer between two Foss instruments for the estragole content in basil oil

Table 1. Range and NIRS correlation statistics for echinacoside content (g/100g) in echinacea roots as well as linalool, estragole and eugenol content (%) in the essential oil of basil.

		spectrometer 1		
analyte	**range**	**mean**	**R^2**	**SECV**
echinacoside	0.098 – 3.327	0.959	0.956	0.169
		spectrometer 2		
echinacoside	0.098 – 3.327	0.959	0.962	0.165
		spectrometer 1		
analyte	**range**	**mean**	**R^2**	**SECV**
linalool	0.20 – 77.37	15.20	0.99	1.03
estragole	0.55 – 98.09	40.03	1.00	0.97
eugenol	0.25 – 66.41	10.02	0.99	0.74
		spectrometer 2		
linalool	0.20 – 77.37	15.20	0.99	1.07
estragole	0.55 – 98.09	40.03	1.00	1.02
eugenol	0.25 – 66.41	10.02	0.99	0.59

Acknowledgements

The financial support of the "Fachagentur für Nachwachsende Rohstoffe" (FNR) in Gülzow, Germany (Grant No.: 98 NR 053) is gratefully acknowledged. Furthermore, the authors would like to thank Bärbel Zeiger, Cornelia Helmund and Christine Langanke for carefully conducting the GC and NIRS measurements.

References

1. H. Schulz, B. Steuer, S. Pfeffer and H. Krüger, *NIR news* **13**, 10 (2002).
2. H. Schulz, in *Near Infrared Spectroscopy in Agriculture*, Ed. by C. Roberts, J. Workman and J. Reeves. A tri-societies monograph (in print).
3. H. Schulz, B. Schrader, R. Quilitzsch, S. Pfeffer and H. Krüger, *J. Agric. Food Chem*. (in print)
4. B. Osborne, T. Fearn and P.G. Randall, *J. Food Technol*. **18**, 651 (1983).
5. J.S. Shenk, M.O. Westerhaus and W.C. Templeton, *Crop Sci.* **25**, 159 (1985).
6. J.S. Shenk and M.O. Westerhaus, *Crop Sci.* **31**, 1694 (1991).
7. P. Tillmann, T.C. Reinhardt and C. Paul, *J. Near Infrared Spectrosc*. **8**, 101 (2000).
8. Y. Wang and B.R. Kowalski, *Appl. Spectrosc*. **46**, 764 (1992).
9. Q. Wang, S. Dejesus, J.P. Conzen, A. Schmidt and H. Weiler, *J. Near Infrared Spectrosc*. **6**, A201 (1998).
10. F. Despagne, D.L. Massart, M. Jansen and H. van Daalen, *Anal. Chim. Acta* **406**, 233 (2000).
11. H. Schulz, S. Pfeffer, R. Quilitzsch, B. Steuer and K. Reif*, Planta Med*. **68**, 926 (2002).