51th Annual Congress of the Society for Medicinal Plant Research

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Can NIR replace complex quantitative methods? Example: *Echinacea* species

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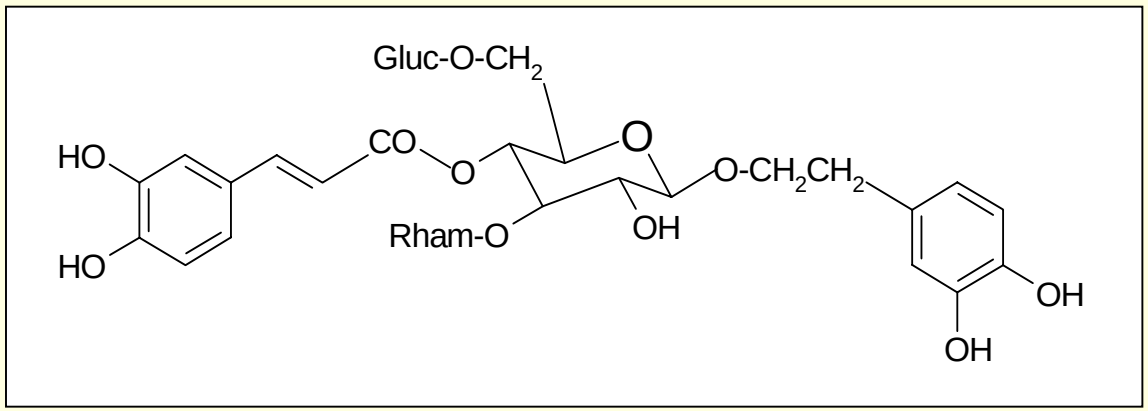
Determination of the echinacoside content in *Echinacea* species by NIRS

Echinacoside content in various *Echinacea* species:

E. purpurea: < 0.01 %
E. pallida: 0.5 - 4 %
E. angustifolia: 0.1 - 2 %



Molecular structure of echinacoside

Determination of the echinacoside content by HPLC

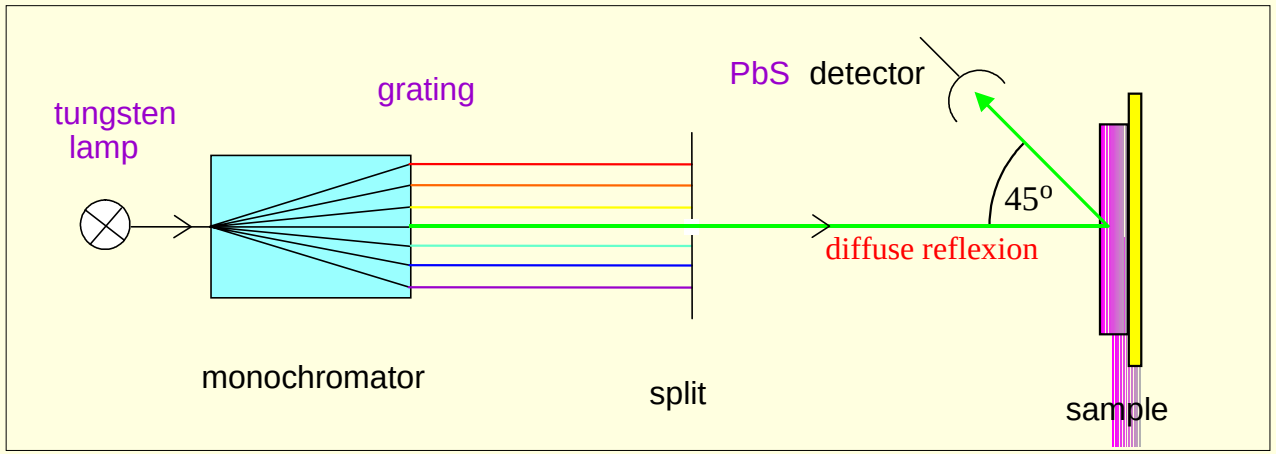
HPLC conditions:

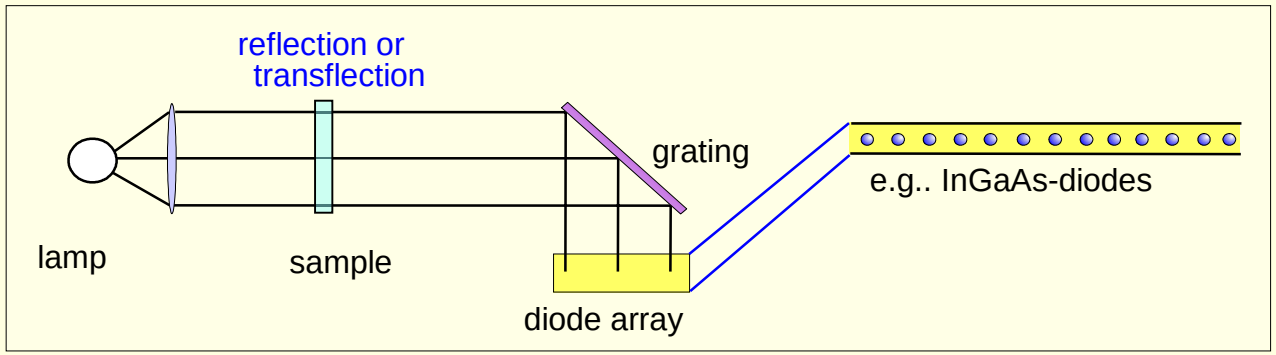
- approx. 1 g powdered drug extracted with 100 mL methanol in a Soxhlet apparatus
- resulting extract evaporated to dryness, residue taken up in 25 mL of the HPLC eluent, centrifugation, injection
- HPLC column: 5µ C18 material, 250 x 4.6 mm i.d.,
  Eluent A: 85 % o-phosphoric acid/water (1:1000 v/v)
  Eluent B: 85 % o-phosphoric acid/acetonitrile (1:1000 v/v)
  Gradient elution programme
- UV-detection: 330 nm
- Quantification according to the method of the external standard

H. Schulz, S. Pfeffer, R. Quilitzsch, B. Steuer, K. Reif, *Planta Medica*. **68**, (2002), 926-929

NIR sample presentation techniques

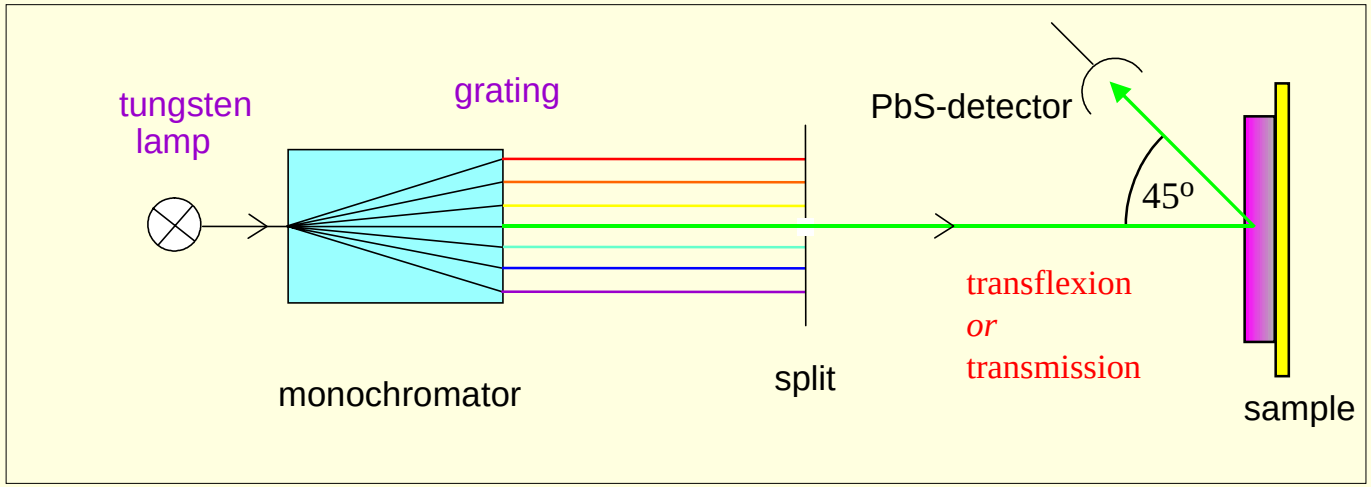
Diffuse reflection measure-
ments in a moving sample cup
with quartz window, 51 x 64 mm



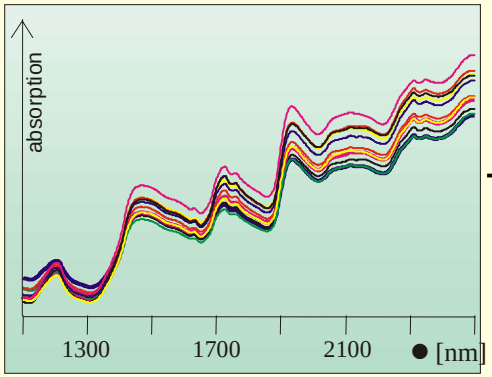


NIR sample presentation techniques

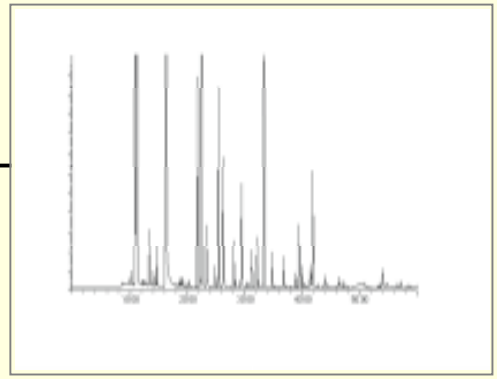
Quartz cell and gold reflector
with defined path length (e.g. 0.2 mm)



Calibration process for quantitative IR, NIR or Raman methods



spectra



reference data

common data set

of spectral and reference data

calibration set

validation set

cross validation

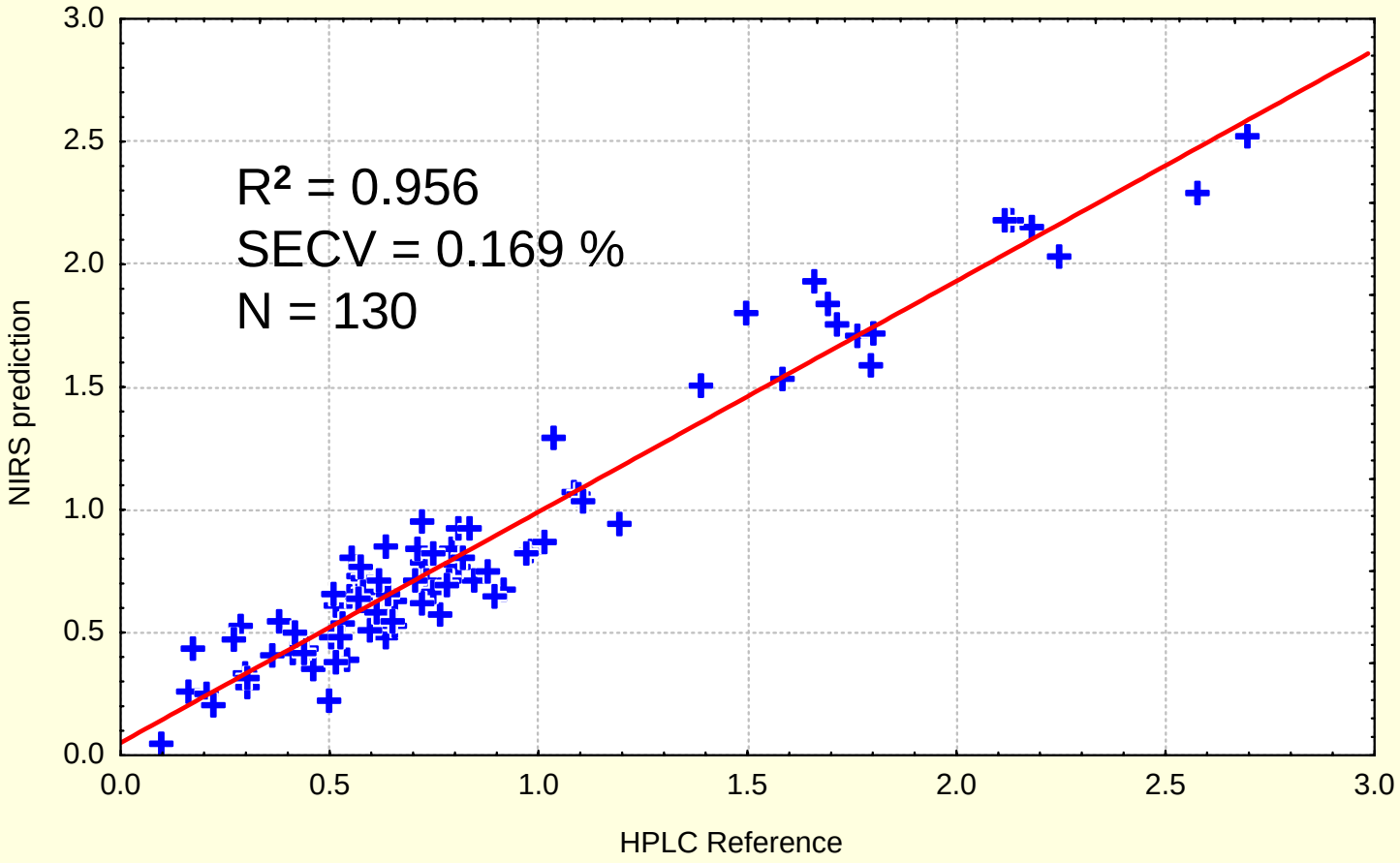
Multiple coefficient of determination (R^2)
Standard error of cross validation (SECV)
Standard error of prediction (SEP)

calibration equation

test

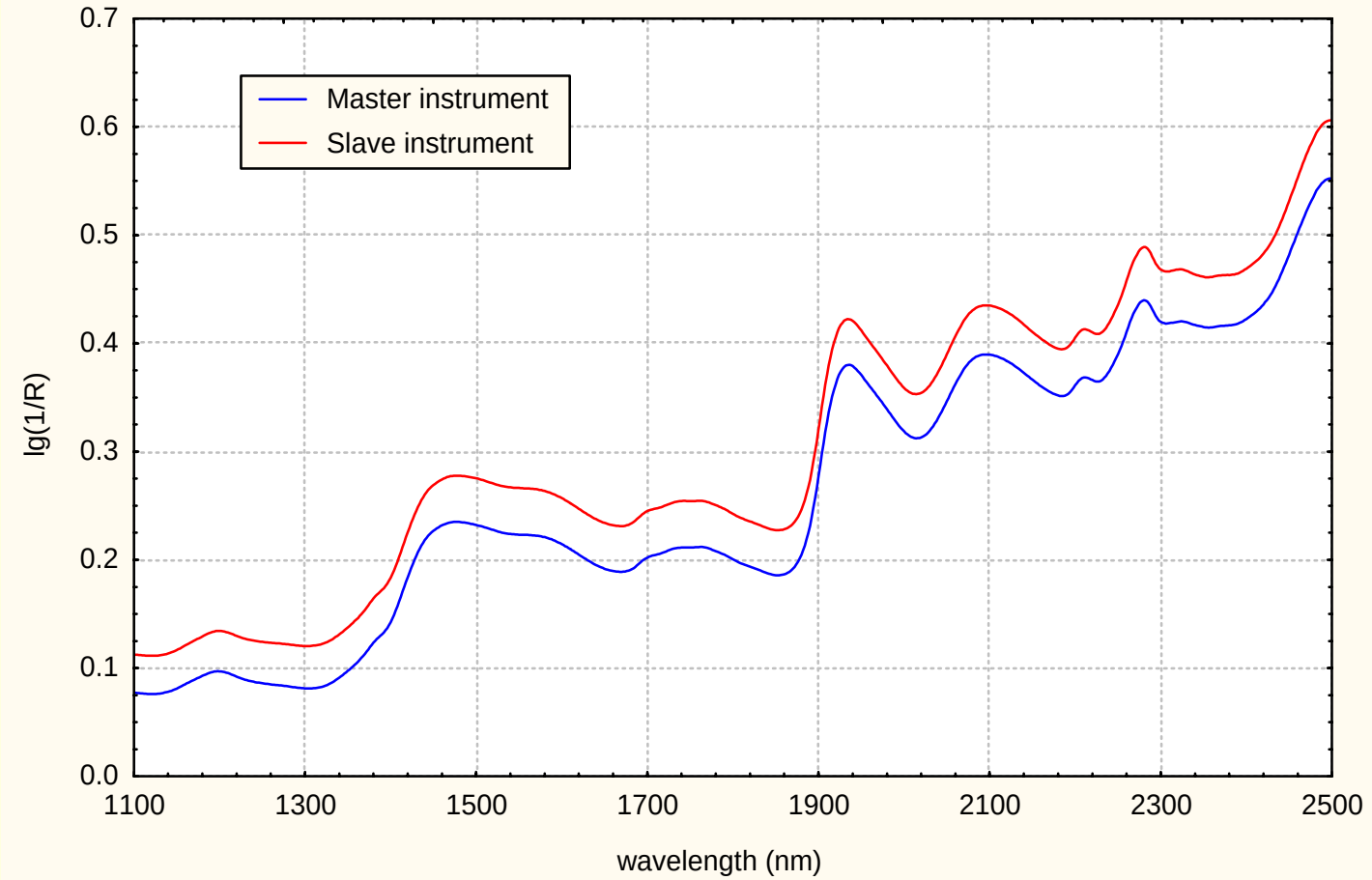
H. Schulz, B. Steuer, H. Krüger, W. Schütze, *Z. Arzn..Gew.Pfl.* **6**, (2001) 138-142

NIRS calibration for echinacoside in *Echinacea* roots



H. Schulz, S. Pfeffer, R. Quilitzsch, B. Steuer, K. Reif, *Planta Medica*. **68**, (2002), 926-929

NIR spectra of the same Echinacea sample measured on two different „NIR Systems 5000“ instruments



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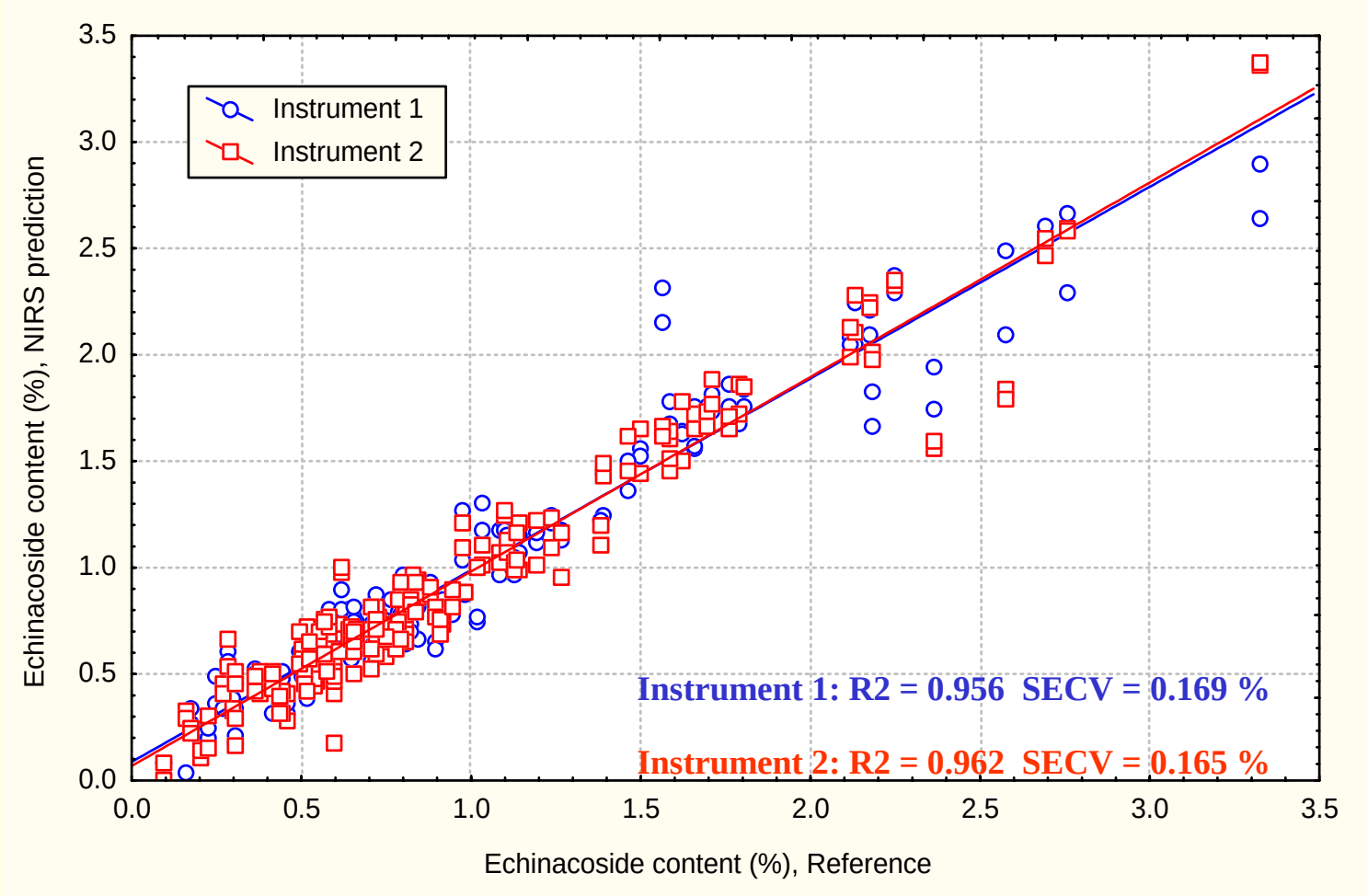
Calibration equations for two „NIRSystem 5000“ instruments (Foss)



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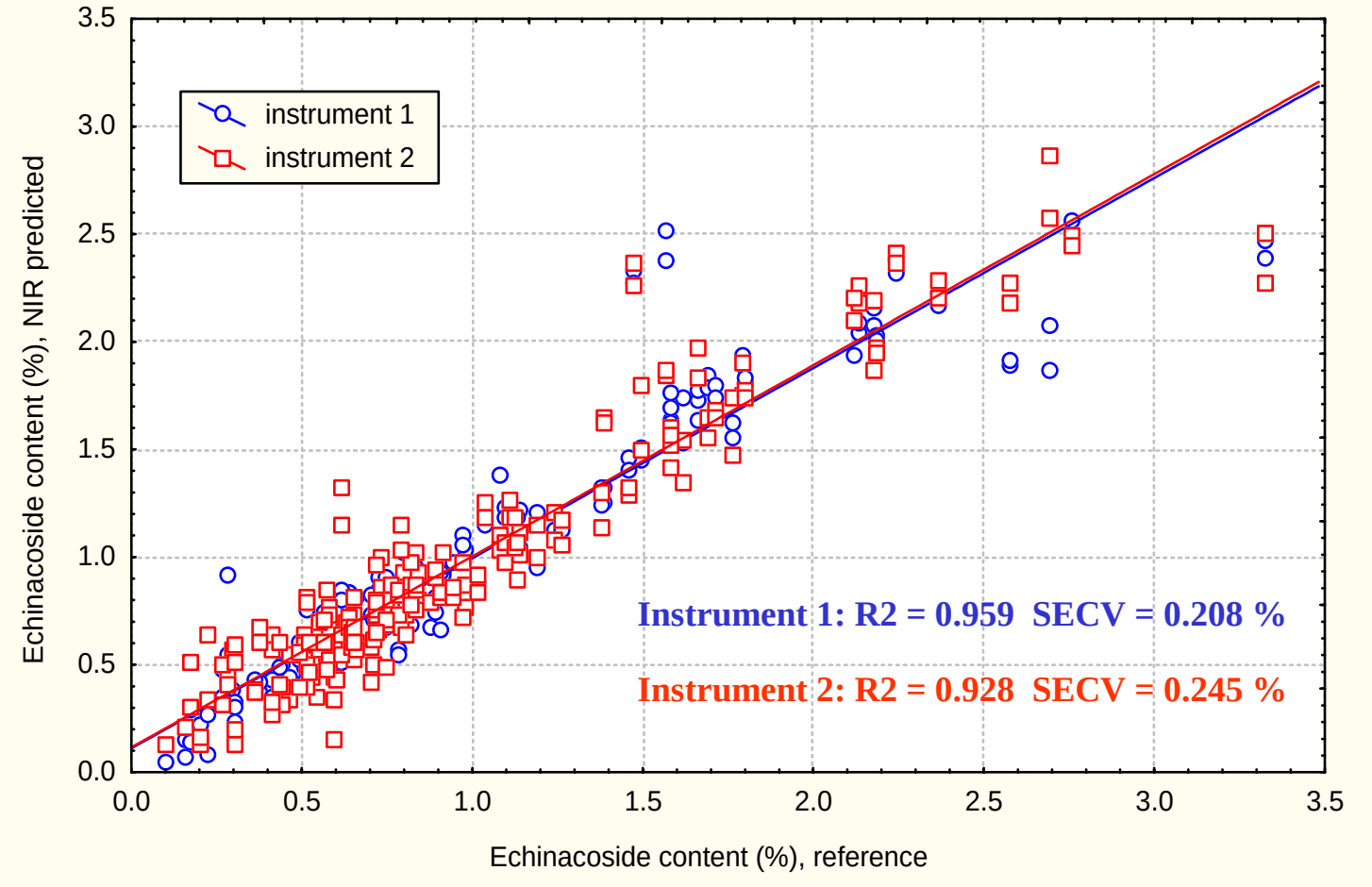
Transfer of spectral data from a „master“ to a „slave“ instrument (Shenk-Westerhaus algorithm)



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Can NIR replace existing quantitative methods ?

- It must be individually checked whether a reliable NIRS prediction is principally possible (calibration model for every sample type!!!)
- Measurement data of several harvests must be considered when establishing a general calibration equation
- There may occur problems when predicting minor components (content < 1 %) not presenting strong specific (N)IR absorptions of the analyt molecules (influence of the matrix)
- Generally, homogeneity may be a problem at some drug samples (solution: if possible, measurements should be performed on powdered samples or at different areas of the inhomogeneous plant material
- Establishing a calibration network only same spectrometer types of the same manufacturer should be used for that purpose

Thank you very much for your attention ! !

Bundesanstalt für Züchtungsforschung an Kulturpflanzen
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