Quantitative Fourier transform near infrared reflectance spectroscopy compared to high performance liquid chromatography of a flavone in *Flos Primulae veris* extracts (Sinupret®)

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Introduction

Sinupret®,[1] an ethanolic extract of five different herbs, one of them *Flos Primulae veris*, is a registered phytopharmacon which is widely used due to its secretolytic, expectorant, antiviral and antiinflammatory activities in order to prevent sinusitis as a consequence of cold. The Substance G4 is determined quantitatively by near infrared (NIR) spectroscopy to control the content of *Flos Primulae veris*. Reversed-phase high-performance liquid chromatography (RP-HPLC) which is routinely used to measure Substance G4 provides the reference data.[2] The method incorporates a time-consuming calibration step. This is compensated by very short analysis times, without sample pretreatment and therefore a large sample throughput can be achieved. 170 NIR spectra of 34 charges were recorded over a wavelength range from 4008 to 9996 cm^{-1}, resolution of 12 cm^{-1} in transflection mode by fibre optics with 10 scans for one average spectrum to equilibrate inhomogeneities. Before chemometric analysis, the NIR spectra were transformed, if necessary, to their derivative spectra (first or second order) to eliminate matrix effects. The quality of each applied chemometric method (PLS or PCR) is characterised by the statistical NIR parameters (r^2, *SEP*, *SEE*, Bias). Optimising the temperature (23°C) and optical thinlayer (0.5 mm) yielded best reproducibility. The presented method is therefore used to raise the efficiency in quality control. The results obtained indicate NIR spectroscopy to be a reliable time-saving alternative method for the routine analysis of the Substance G content in *Flos Primulae veris* in the phytopharmaceutical industry.

NIR spectroscopy is a powerful tool for determination of bioactive substances in various agricultural products. Whereas it is used mostly to determine major constituents such as proteins, fats, carbohydrates or water, in the last years NIR spectroscopic determinations of some minor constituents have also been described in the literature.[3] Therefore this study is aimed at establishing a new and rapid method for determination of a flavone in *Flos Primulae veris* extracts.

Method

The HPLC method for determination of the flavone Substance G in *Flos Primulae veris* (run time 40 min) is used as reference method.Without any pretreatment the samples were scanned directly with the NIR spectrophotometer (Bühler AG, Uzwil, Switzerland), from 4008 to 9996 cm^{-1} in transflection mode (measurement time: approximately 30 s for 10 scans). The spectral analysis program (bcap 6.00, Bühler Analytical

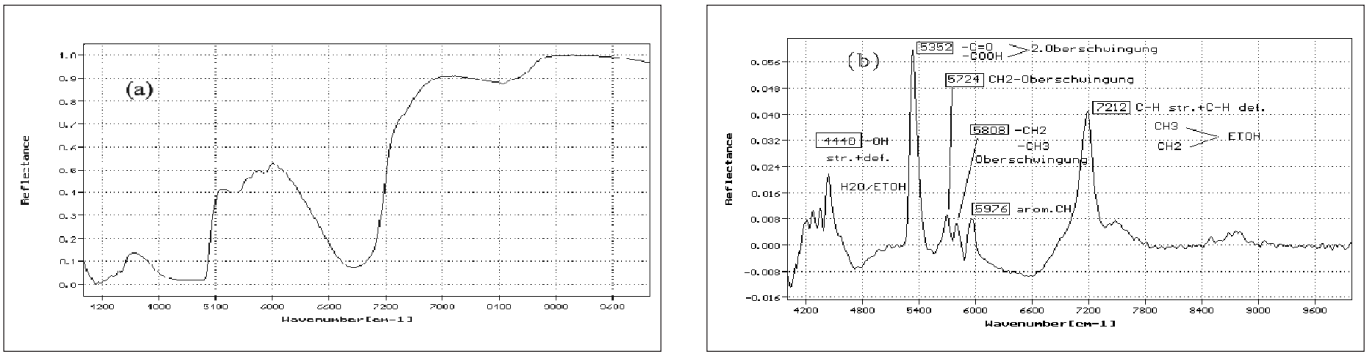

Figure 1. (a) NIR spectra of Sinupret; (b) characteristic frequencies.

Table 1. NIR calibration (15 factors) and validation statistics for flavone in Sinupret.

Data pretreatment	normalise	1st deriv.	Normalise & 1st deriv.	1st deriv. & norm.	2nd deriv.	absorb.
r^2	0.9558	0.9538	0.9564	0.9594	0.9398	0.8989
SEP	0.0164	0.0113	0.0144	0.0099	0.0209	0.0129
Bias	–0.0261	–0.0455	–0.0423	–0.0389	–0.0396	–0.0052
SEE	0.0078	0.0051	0.0051	0.0057	0.0073	0.0133

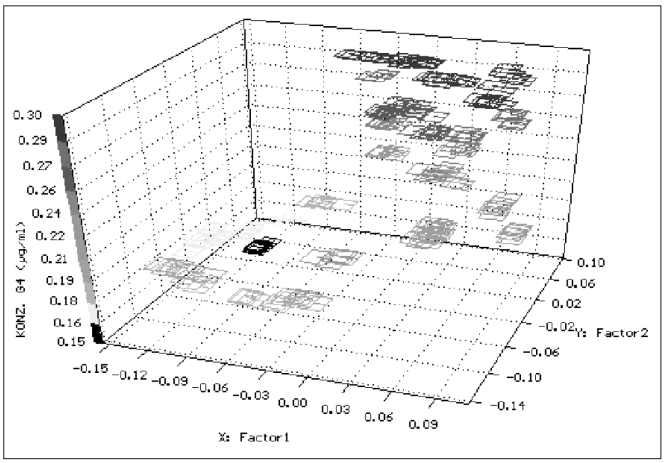

Figure 2. Principal Component Analysis (PCA) of NIR data for different Sinupret charges.

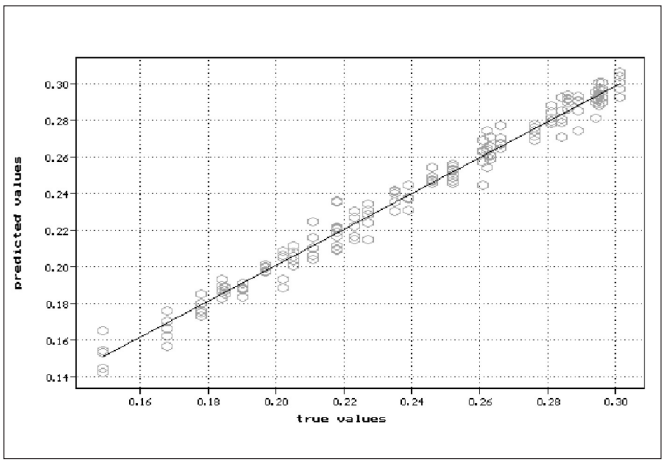

Figure 3. NIR prediction versus reference determination (RP-HPLC) of G4-content in Sinupret in the calibration data set.

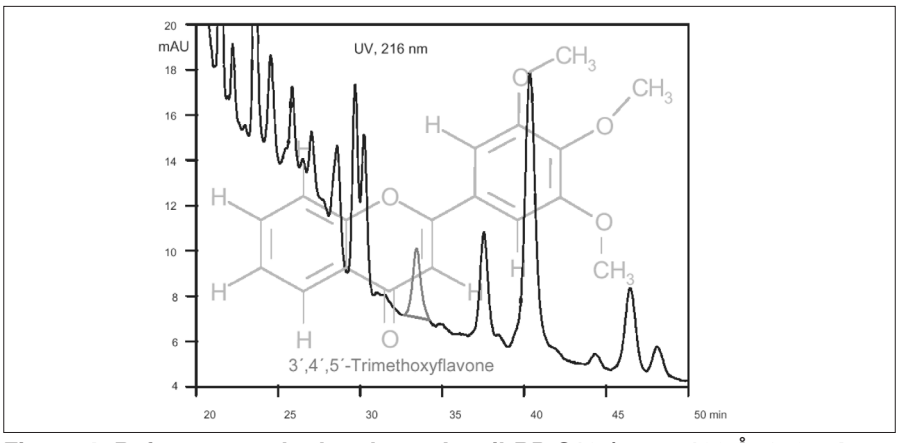

Figure 4. Reference method: column, Inosil RP-C18 (5 µm, 100 Å, 250 × 4 mm ID), mobile phase, (A) water, (B) acetonitrile, gradient, 0–38% B in 10 min, 38% B for 45 min, 38–100% B in 2 min; temperature 21°C, flow rate, 1 mL min^{-1}; detection, UV, 216 nm.

Chemical Package, Bühler AG, Uzwil, Switzerland) was used to process the data and to develop the most appropriate chemometric method. The calibration program was set up with the full wavelength range using the PLS (Partial Least Square) algorithm (Figure 3). The individual error of prediction value is described by the standard error of prediction (*SEP*), the relationship between reference method and NIR analysis by the multivariate coefficient of determination (r^2) (Table 1).

Results and discussion

The received results, presented in Figure 3 and Table 1, demonstrate that NIR technique is well suited for the non-destructive and reliable determination of minor compounds in phytopharmaca. Best statistical results for this procedure were received by 1st derivative/normalise as datapretreatment (15 Principal Components, *SEP* = 0.00989, *r* = 0.9594).With this procedure it is possible to predict the content of the flavone after an analysis time of approximately 30 s, whereas the usual analytical chromatography methods (e.g. HPLC) include time consuming sample clean-up procedures. The described technique is of practical interest for quality control, but also for studies of growth in the field, the maturity stage and the best harvest-time.

References

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2. C.W. Huck, *Isolierung, Analytk und Strukturaufklärung neuer Wirkkomponenten in* Primula veris. Thesis, University of Innsbruck (1998).
3. H. Schulz, *Beiträge zur Züchtungsforschung* **2,** 347 (1996).