Quality control of liquid plant extracts in the phytopharmaceutical industry

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Introduction

Near infrared (NIR) reflectance spectroscopy plays an increasing role in manufacturing to improve quality and process efficiency. NIR can be used for on-line quality control of liquid *Flos Primulae veris*[1] extracts. NIR reflectance spectroscopy is a fast and reliable method, supported by highly sophisticated statistical software, to perform principal component regression (PCR) or partial least square regression (PLSR).[2] NIR reflectance spectroscopy is a non-destructive method that offers the possibility of measuring physical and chemical properties at once. For a reliable calibration a wide range of laboratory and production samples should be used. Methoxyflavones are known to possess anti-allergic, anti-inflammatory, anti-viral, anti-proliferative and anti-carcinogenic activities and they also effect some aspects of mammalian metabolism.[3] In this work an NIR method for the determination of the leading compound 3´,4´,5´-trimethoxyflavone, in order to control the content of *Flos Primulae veris*, is presented and compared to HPLC analysis. Furthermore, the water- and ethanol-content of the liquid plant extract is determined.

Materials and methods

Forty four charges of liquid plant extracts were received from Herbextracts S.L., Calle Ramón Llull 6, 07330 Consell, Mallorca, Spain.

RP-HPLC

All charges were determined three-fold and cross-wise on a LCM 1 Module (Waters, Millford, MA, USA). For the analysis of 3´,4´,5´-trimethoxyflavone on a 250 × 4 mm i.d. column (Inosil 100 RP18, 5 µm, 100 Å, Innovex, Vienna, Austria), an aqueous gradient up to 38% acetonitrile within 10 min, followed by an isocratic hold at 38% acetonitrile for 45 min and a linear gradient up to 100% acetonitrile within 5 min was used. Flow rate, 1 mL^{-1}. Temperature, 21°C. Detection, UV, 216 nm. Sample size, 100 µL.

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GC-FID

HP1 fused silica (50 × 0.32 mm i.d.). Gas, hydrogen 0.7 bar. Injector temperature, 30°C. Temperature from 50°C (4 min) up to 220°C (8°C min^{-1}) and up to 300°C (20°C min^{-1}). Detector temperature, 300°C. Split, 35 mL^{-1}:120. Sample size, 5 μL.

Karl-Fischer titration

20 μL of each charge were titrated on a 684 KF Coulometer (Metrohm, Filterstadt, Germany).

NIR reflectance spectroscopy[4]

220 NIR spectra of 44 charges were recorded with an FT-NIR spectrometer (Buehler, Uzwil, Switzerland) 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 eliminate inhomogenities.

Results and discussion

After the content of the leading compound 3´,4´,5´-trimethoxyflavone was determined by RP-HPLC[4] the temperature and the optical thin layer had to be optimised. Mathematical pretreatment and statistical analysis were carried out by performing partial least square regression (PLSR). Recording the NIR spectrum [Figure 1(a)] and calculation of the first derivative spectrum [Figure 1(b)] allowed the identification of characteristic absorption bands. The most intense band in the spectrum belonged to the vibration of the second overtone of the carbonyl goup (5352 cm^{-1}). All recorded spectra were normalised (i.e. Ordinate values are stretched between zero and one) and transformed to their first derivative before calculating in the linear PLS model. Normalisation allowed the baseline shift to be minimised.[5] Fifteen principal components were necessary in order to reach the best calibration equation (Figure 2). The robustness of the NIR reflectance spectroscopy model is high, which is demonstrated in the similarity of the results for *SEE* (0.0057) and *SEP* (0.0099). Accuracy is expressed in the bias. Its value is –3.89% with respect to the mean. This means that the RP-HPLC results agree with NIR reflectance spectroscopy on average. A study of the temperature influence showed that a rise in the temperature induces a broadening in fundamental vibrational bands. For recording of the NIR spectra all samples were thermostatted at 23°C in a stirred water bath. The influence of the optical thin layer on the first derivative of the reflectance for the second overtone of the carbonyl function showed maximal transmission at a pathway length of 0.5 mm. Finally a correlation coefficient of 0.95421 for

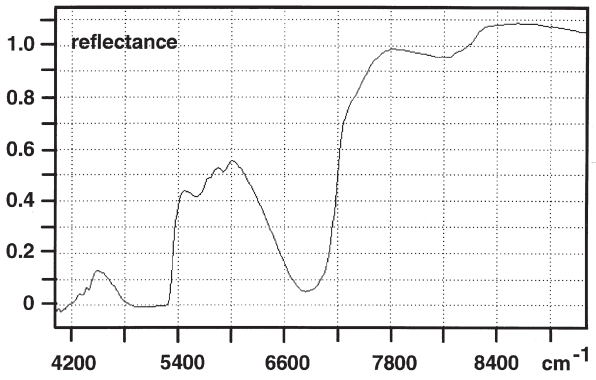

Figure 1(a). NIR spectrum of a *Flos Primulae veris* extract.

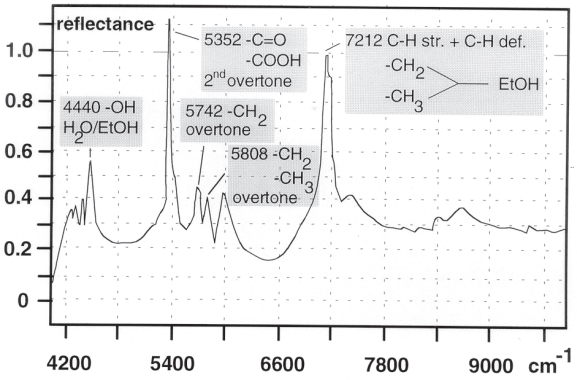

Figure 1(b). First derivative of the NIR spectrum with characteristic vibrations.

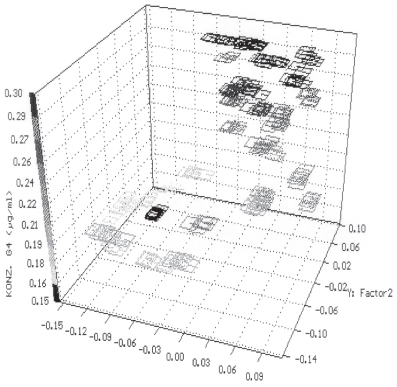

Figure 2. 3D-factor-plot of the *Flos Primulae veris* extract.

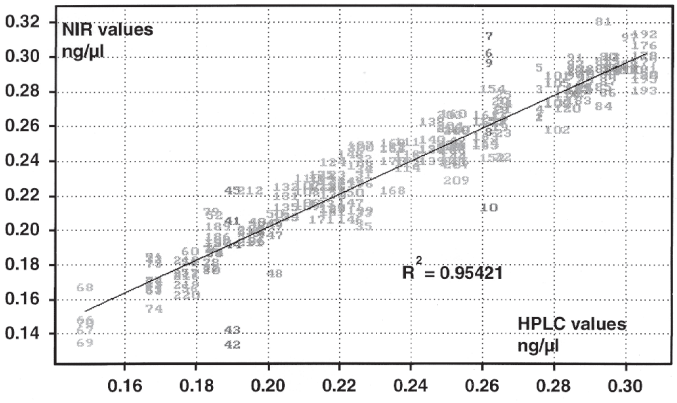

Figure 3. Calibration curve for 3´,4´,5´-trimethoxyflavone. Correlation between RP-HPLC and NIR reflectance spectroscopy.

the calibration curve of NIR values against HPLC values helped to assess the linearity of the model (Figure 3). This means that the leading compound 3´,4´,5´-trimethoxyflavone can be determined by NIR reflectance spectroscopy with high precision in the lower ppm range between 0.14 and 0.32 ppm.

For the control of the solvent composition, a calibration curve with a correlation coefficient of 0.99530 for the determination of the water content was calculated (Figure 4). Therefore, the reference data were received by Karl-Fischer titration. In the same way, also, the correlation of the ethanol content between the gas chromatographic and the NIR reflectance spectroscopy method showed a correlation coefficient of 0.99701 (Figure 5).

Finally, validation of the NIR reflectance spectroscopy model showed that the robustness for the determination of the 3´,4´,5´-trimethoxyflavone, water and ethanol content is high. Nevertheless, it has to be noticed that NIR reflectance spectroscopy is an analytical method that is never fully enough

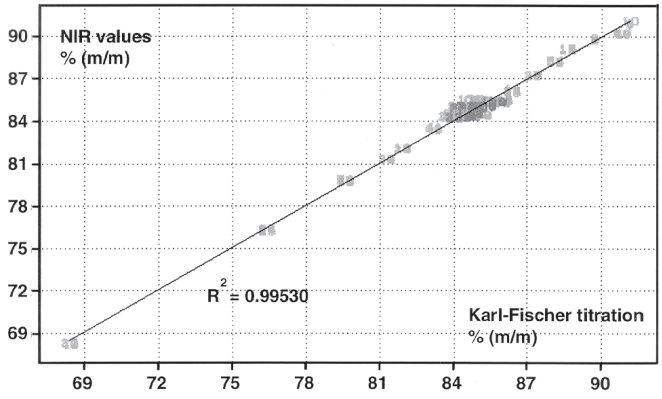

Figure 4. Calibration curve for water. Correlation between Karl-Fischer titration and NIR reflectance spectroscopy.

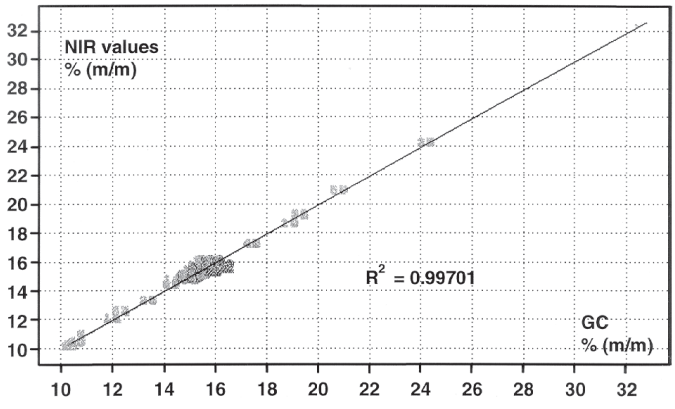

Figure 5. Calibration curve for ethanol. Correlation between gas chromatography and NIR reflectance spectroscopy.

even though the first calibration model works satisfactorily. It is necessary to collect additional samples especially when their matrix properties are not included in the sample collection.

Even though costs for the equipment are high and calibration needs a lot of time, NIR reflectance spectroscopy has the great advantage of reducing time and costs, as the solvent consumption is virtually zero. Because of the short analysis time of *c*. three seconds compared to 50 minutes by RP-HPLC, a high sample throughput is guaranteed. Furthermore, physical and chemical properties can be determined at once.

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